TOXICOLOGICAL REVIEW

Phenol

(CAS No. 108-95-2)

October 2000

In Support of Summary Information on

Integrated Risk Information System (IRIS)

NOTICE

This document is a **preliminary draft**. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for peer review on its technical accuracy and policy implications.

U.S. Environmental Protection Agency
Washington D.C.

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This document is a preliminary draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

Foreword

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response information in IRIS pertaining to chronic exposure to phenol. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of phenol.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. EPA, 1995a). Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development, Office of Air and Radiation, Office of Prevention, Pesticides, and Toxic Substances, Office of Solid Waste and Emergency Response, Office of Water, Office of Policy, Planning and Evaluation and the Regional Offices.

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1. INTRODUCTION

This document presents the derivation of the noncancer dose-response assessments for oral exposure (the oral reference dose or RfD] and for inhalation exposure [the inhalation reference concentration or RfC), and the cancer hazard and dose-response assessments.

The RfD and RfC are meant to provide information on long-term toxic effects other than carcinogenicity. The reference dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation reference concentration (RfC) is analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is expressed in units of mg/m^3.

The carcinogenicity assessment is meant to provide information on three aspects of the carcinogenic risk assessment for the agent in question: the U.S. EPA classification, and quantitative estimates of risk from oral exposure and from inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for phenol has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment include the following: The Risk Assessment Guidelines (U.S. EPA, 1987), the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), (proposed) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), (proposed) Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1995b), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988) and the Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995c), Guidance on Risk Characterization (U.S. EPA,1995a), and Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b).

Literature search strategy employed for this compound were based on the CASRN and at least one common name. As a minimum, the following databases were searched: RTECS, HSDB,

TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE AND MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

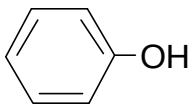
2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Phenol is a monosubstituted aromatic hydrocarbon. In its pure state, it exists as a colorless or white solid. This pure compound is mixed with water and commercially sold as a liquid product. Phenol gives off a sweet, acrid smell detectable to most people at 40 ppb in air, and at about 1-8 ppm in water (ATSDR, 1998). It evaporates more slowly than water and is moderately soluble in water. Phenol is also combustible.

Phenol is produced through both natural and anthropogenic processes. It is naturally occurring in some foods, in human and animal wastes, in decomposing organic material, and is produced endogenously from the metabolism of protein. Phenol has been isolated from coal tar, but it is now synthetically manufactured. Currently, the largest use of phenol is as an intermediate in the production of phenolic resins, which are used in the plywood, adhesive, construction, automotive, and appliance industries. Phenol is also used in the production of synthetic fibers, such as nylon, and for epoxy resin precursors such as bisphenol-A. Phenol is toxic to bacteria and fungi, and is used as a slimicide and disinfectant. Because of its anesthetic effects, phenol is used in medicines such as ointments, ear and nose drops, cold sore lotions, throat lozenges, and antiseptic lotions.

The greatest potential source of exposure to phenol is in the occupational setting, where phenol is used in manufacturing processes. People are also exposed in consumer products such as medicines and lotions, as well as in some foods and in tobacco smoke. Phenol has been found in drinking water.

Table 1. Physical Properties and Chemical Identity of Phenol

CAS Registry Number	108-95-2	Lide, 1993
Synonym(s)	Benzenol, hydroxybenzene, monophenol, oxybenzene, phenyl alcohol, phenyl hydrate, phenyl hydroxide	ATSDR, 1998
Registered trade name(s)	Carbolic acid, phenic acid, phenic alcohol	ATSDR, 1998
Melting point, C	43	Lide, 1993
Boiling point, C	181.8	Lide, 1993
Vapor pressure, at 25 C	0.3513	HSDB, 1998
Density, at 20 C relative to the density of H_2O at 4 C	1.0576	Lide, 1993
Flashpoint (open cup)	85 C	ATSDR, 1998
Water solubility, g/L at 25 C	87	Lide, 1993
Log K_{OW}	1.46	HDSB, 1998
Odor threshold	0.047 ppm (0.18 mg/m^3) - 100% response 0.006 ppm (0.02 mg/m^3) - sensitive	U.S. EPA, 1986
Molecular weight	94.12	Calculated
Conversion factors	1 ppm (v/v) = mg/m^3 x 0.260 1 mg/m^3 = ppm (v/v) x 3.85	Calculated
Empirical formula	C_6H_6O	Lide, 1993
Chemical structure		Not applicable

3. TOXICOKINETICS/TOXICODYNAMICS RELEVANT TO ASSESSMENTS

Phenol is readily absorbed by the inhalation, oral, and dermal routes. Portal of entry metabolism for the inhalation and oral routes appears to be extensive, and involves sulfate and glucuronide conjugation, and, to a lesser extent, oxidation. The primary oxidative metabolites include hydroquinone and catechol, which are also substrates for conjugation. Secondary products of hydroquinone or catechol, which include benzoquinone and trihydroxybenzene, can also be formed. Phenol is widely distributed in the body, although the levels in the lung, liver, and kidney are often reported as being higher than in other tissues (on a per gram tissue basis). Elimination from the body is rapid, primarily as sulfate and glucuronide conjugates in the urine, regardless of route of administration. Phenol does not appear to accumulate significantly in the body.

3.1 Absorption

Extensive absorption following inhalation exposure has been demonstrated in both studies in humans and in laboratory animals. Piotrowski (1971) studied lung and skin absorption of phenol in human volunteers exposed to air concentrations of 6 to 20 mg/m^3 for 8 hours. In the lung absorption studies, the subjects inhaled phenol through a face mask, eliminating the potential for most dermal absorption. These subjects retained 60-88% of the inhaled phenol, and the percent retained did not vary with exposure concentration. The absorption rate leveled off after approximately 3 hours of exposure, indicating that absorption had reached steady state. In the skin absorption studies, subjects wore underwear and denim coveralls or were unclothed for different trials of the experiment and in each case were supplied with fresh air from outside the chamber for breathing. The absorption coefficient did not appear to vary greatly with exposure for 6 hours to concentrations in air ranging from about 5 to 25 mg/m^3 and clothing did not appear alter the absorption rate. The mean absorption coefficient was 0.35 m^3/hr, indicating that the amount of phenol present in 0.35 m^3 of air was absorbed through the skin per hour. The quantitative data from the dermal exposure study are, however, limited for risk assessment purposes, due to the short duration of the exposure and the absence of a direct determination of whether the absorption rate had reached steady state.

Other studies of workers exposed to phenol provide evidence for significant absorption via the inhalation route; however, the contribution of dermal absorption from direct contact with liquid phenol or phenol in air was not assessed in these studies. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. The total and conjugated phenol levels tended to increase with increasing air concentration, but free phenol levels were not affected. This suggests that at the concentrations studied (up to 12.5 mg/m^3), phenol conjugation was not rate limiting. The study authors did not evaluate specifically the levels of oxidative phenol metabolites, so no conclusion can be made regarding whether Phase I metabolism was rate limiting. Based on mass balance analysis, the authors concluded that phenol is efficiently absorbed from the lung, since the phenol dose (air concentration * air volume/hour) was similar to the total mass excreted in the urine. Other occupational studies provide qualitative evidence for lung absorption, reporting increasing urinary excretion of phenol metabolites with increasing workplace air concentrations. In many cases, the data are not adequate to estimate the rate and

degree of absorption through this route, and potential contributions of dermal absorption are often inadequately described (Ogata et al., 1986; ACGIH, 1996).

Absorption through the lung has also been evaluated in laboratory animal studies following inhalation exposure or intratracheal administration. In an unpublished study, Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in Fischer 344 rats following inhalation exposure to 25 ppm (96 mg/m^3) for 6 hours (nose-only).[1] Radioactivity in the blood was at steady state levels at the first measured time point (120 minutes after beginning the 6-hour exposure), indicating rapid absorption kinetics. Hughes and Hall (1995) evaluated the disposition of phenol following intratracheal and intravenous (i.v.) administration of 63.5 nmol of ^{14}C-phenol to female Fisher 344 rats. The recovery of radioactivity in tissues and excreta for both routes was approximately 90% of the administered dose within 72 hours. Since the amount of radioactivity recovered was nearly equal for the intratracheal and i.v. dose routes (and fecal excretion is minimal), the authors concluded that absorption was near 100%. Hogg et al. (1981) administered ^{14}C-phenol intratracheally in isolated perfused rat lungs from MRC hooded rats. At the end of the experiment (perfusions were approximately 85 minutes), approximately 92% of the administered radioactivity was in the perfusate, 6% was recovered in the lung lavage, and approximately 3% was associated with lung tissue. The high recovery in the perfusate indicated that phenol is nearly completely absorbed across the airways.

Human evidence for oral absorption indicates rapid and complete absorption. In a study of three human volunteers, Capel et al. (1972) found that 85-98% of a 0.01 mg/kg oral dose of ^{14}C-phenol was excreted in the urine within 24 hours. In addition, case reports of oral poisoning provide qualitative evidence for gastrointestinal absorption of phenol, but the ingested and absorbed dose were not estimated in these reports, and in some cases both oral and dermal exposure were involved (Tanaka et al., 1998).

Numerous laboratory animal studies found that orally administered phenol is readily absorbed. In most cases, absorption rates were not calculated, but the rapid recovery of high percentages of administered doses in the urine, with only minimal recovery in feces and tissues, demonstrated nearly complete absorption. In various studies in the rat, the percentage of the administered dose recovered ranged from 65 to 96.5 percent over a wide range of doses (Kao et al., 1979; Edwards et al., 1986; Kenyon et al., 1995). Varying degrees of absorption have been observed in a variety of other species. In a study of 18 animal species orally administered single doses ranging from 20-50 mg/kg ^{14}C-phenol, Capel et al. (1972) found that the percentage of radiolabel recovered in the urine varied from 31% of the administered dose in squirrel monkeys to 95% in Wistar rats in 24 hours. It is not clear, however, if these differences represent differences in the degree of absorption or in the rate of elimination. Hughes and Hall (1995) found that in female F344 rats administered 63.5 nmol of ^{14}C-phenol by oral gavage, total recovery of radioactivity (in tissues and excreta) was approximately 90 percent of the administered dose

[1]This study has not been peer-reviewed, but it was well-conducted according to EPA guidelines for a pharmacokinetics study (with minor deviations).

within 72 hours. Since fecal excretion is approximately 1-3%, and the recovered dose was nearly equal for oral and i.v. dose routes, the authors concluded that the absorption was near 100%. The difference between the 90% recovery and 100% total absorption was attributed to losses that were consistent across doses.

Rapid absorption of orally administered phenol has been observed in a number of studies. Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in Fischer 344 rats following oral dosing by gavage or in drinking water. Recovery of the administered dose was approximately 95%, regardless of the dosing protocol. In the high-dose gavage animals (150 mg/kg), peak levels of radioactivity in blood were detected 15 minutes post administration, indicating rapid uptake kinetics. Humphrey et al. (1980) found that free phenol levels in the plasma of rats given an oral dose of 300 mg/kg radiolabeled phenol reached a maximum of 26 g/mL at the first measured time, about 10 minutes after dosing, and declined rapidly to background by 60 minutes. They observed similar results in Beagle dogs given a 40 mg/kg dose, with rapid peak levels of 7.8 g/mL and complete removal of free phenol by 1 hour.

More quantitative kinetics data are available from *in situ* perfusion studies. Humphrey et al. (1980) administered ^{14}C- phenol (1 mg/mL) to the gut lumen of rats by means of a duodenal cannula, and the remaining radioactivity was measured over 30 minutes, at 3-minute intervals, in perfusate collected by an ileal cannula. The results from the intestinal perfusion studies indicated that removal of ^{14}C-phenol obeys first-order kinetics with a lumenal $T_{1/2}$ of 5.5 minutes and rate constant for absorption of 0.127 min^{-1}. These authors also measured the plasma concentrations of phenol in the portal vein and posterior vena cava of dogs following intraduodenal dosing with either 40 or 160 mg/kg phenol. At either dose, the concentration was already maximal in the portal vein plasma within 3 minutes after dosing (the first measurement taken) and had decreased to non-detectable levels within 1 hour at the low dose and to 33% at the high dose. These data show that in both species phenol is rapidly absorbed from the gut. Powell et al. (1974) added ^{14}C-phenol to the mucosal medium of isolated rat gut preparations, and measured the level of radioactivity in the mucosal and serosal medium over 2 hours. They found that 78% of the administered radiolabel had been transferred to the serosal medium over this period. Kao et al. (1979) administered ^{14}C-phenol (12.5 or 25 mg/kg) to rats intra-duodenally. Recovery of the radioactivity was rapid, with over 70% recovered in the urine within 2 hours.

The dermal route of exposure is an important one. Both absorption of phenol liquid directly in contact with skin, and dermal absorption from exposure to phenol vapor are of concern. Significant dermal absorption of phenol can result from phenol in air (Piotrowski, 1971). Based on an analysis of the Piotrowski (1971) data, ATSDR (1998) concluded that over air concentrations ranging from 5-25 mg/m^3, the amount of phenol absorbed through the skin will be about half of that absorbed through the lungs. The conclusion was reached by estimating the amount of phenol absorbed through the lung as the product of the human ventilation rate of 0.8 m^3/hour and the steady state lung retention fraction of 0.7 reported by Piotrowski (1971). The resulting lung absorption coefficient of 0.6 m^3/hour is nearly twice the skin absorption coefficient of 0.35 m^3/hr. This analysis is limited, however, because it is not clear that the exposure duration was long enough for steady state to be reached in the dermal absorption studies. Absorption via

the dermal route may be lower at steady state, due to the potential for a back-pressure from phenol levels in blood.

A number of case reports and *in vitro* studies have resulted in quantitative estimates of phenol absorption through the skin. Bentur et al. (1998) reported an accidental dermal poisoning case in which a solution of 90% phenol was spilled on the left foot (3% of body surface). The exposure site remained occluded and no attempt at decontamination was made until the onset of symptoms that began within 4.5 hours. Following admission to the hospital shortly afterwards, peak serum phenol levels of 21.6 g/mL were measured. Baranowska-Dutkiewicza (1981) applied a reservoir of 2.5, 5, or 10 g/L phenol solution on a small area of the forearm of 12 male volunteers. The absorption rate was dependent on the concentration, and ranged from 0.08 mg/cm^2/hour at the low concentration, to 0.301 mg/cm^2/hour at the high concentration. At the low concentration, the total amount of phenol absorbed, but not the absorption rate, increased with increased time; approximately 13% of the applied dose was absorbed over a 30-minute period. In an *in vitro* study, 20% of applied doses ranging from 1.3 to 2.7 g/cm^2 were absorbed from unoccluded human skin patches within 72 hours, while addition of a Teflon cap resulted in 47% absorption over this same period (Hotchkiss et al., 1992).

The ability of phenol to be absorbed through the skin has also been evaluated in laboratory animals. Hughes and Hall (1995) administered 63.5 nmol of labeled phenol to an occluded dermal patch (2.5 cm^2) of female F344 rats. Maximal recovery of the radioactivity was approximately 70%. The site of dermal application was washed 72 hours post treatment and yielded 14% of the recovered dose; 1.6% of the recovered dose was present in the skin at this site. Thus, approximately 15% of the dose was not absorbed within 72 hours. In an *in vitro* study, (Hotchkiss et al., 1992) found that phenol absorption by rat skin is similar to that of human skin, and was approximately 20-50% in 72 hours, depending on the conditions.

Taken together, the human and laboratory animal data demonstrate that phenol is readily absorbed following exposure by all dose routes. The recovery of greater than 90% of the administered phenol dose as urinary metabolites provides direct evidence that the administered dose was nearly completely absorbed. The route of administration appears to play a limited role, with skin absorption reported as less extensive than absorption from the lung or gut. In most studies, absorption rate constants have not been calculated; however, the measurement of peak blood phenol concentrations within minutes of dosing indicates that absorption is rapid.

3.2 Distribution

Studies in humans and laboratory animals indicate that phenol is widely distributed throughout the body regardless of exposure route. Since phenol is rapidly excreted, studies on tissue distribution of phenol typically evaluate only a small fraction of the absorbed dose.

Several fatal poisoning case studies evaluated phenol concentrations in multiple tissues (Tanaka et al., 1998). Generally, phenol is widely distributed. Higher tissue concentrations relative to blood have been reported for some organs, particularly for the liver and kidneys, although this finding has not been reported consistently across all studies.

Morrison et al. (1991) reported on the kinetics of phenol injected intramuscularly in a motor point block procedure in pediatric patients. Administered doses ranged from 6.7 to 70 mg/kg and blood phenol concentration was measured at 5, 15, 30, 60, and 120 minutes after the last injection. Phenol reached peak levels 5 to 15 minutes after administration and rapidly declined to 3 to 34 percent of peak levels within 120 minutes. Peak phenol concentration (g/mL) in blood as a function of administered dose (mg/kg) was determined ($y = 0.483x - 3.244$; $r = 0.873$). Pretreatment levels of blood phenol ranged from 0.3 to 0.8 g/mL.

The laboratory animal data provide additional evidence for elevated tissue concentrations in the lung, liver, and kidney, although the magnitude of the tissue differences varies from study to study. Liao and Oehme (1981) evaluated the tissue distribution of 207 mg/kg ^{14}C-phenol orally administered to male Sprague-Dawley rats. Total radioactivity in tissues declined rapidly from a maximum of 28.4% of the administered dose at 0.5 hours, to 16.6% at 1 hour and 0.3% at 16 hours. Tissue concentrations measured at time points between 0.5 and 16 hours were significantly greater than in plasma for the liver, spleen, kidney, and adrenal gland; lungs and thyroid were also marginally elevated. The liver had the greatest amount of phenol, accounting for 29- 56% of the total radioactivity recovered from tissues at the various time points. The study authors attributed the high levels in the liver to both an elevated tissue concentration, and the large relative organ size. Dow Chemical Co. (1994) conducted a study of ^{14}C-phenol administered to Fischer 344 rats by oral gavage of 1.5, 15, or 150 mg/kg; in drinking water at 5000 ppm; or via nose-only inhalation at 25 ppm for 6 hours. Tissue levels were measured in the kidneys, liver, lung, muscle, skin, spleen, testes, ovaries and carcass 24 hours after exposure by the various routes. The only sites with a statistically significant increase in phenol levels were the kidney and liver (5- to10-fold higher levels than other tissues); this finding was consistent across dosing regimens. Hughes and Hall (1995) evaluated the disposition of radiolabeled phenol administered dermally, by oral gavage, i.v., or intratracheally to female F344 rats. When the rats were sacrificed 72 hours after administration by any of these four routes, tissue concentrations represented only 1-5% of the recovered dose. No tissue appeared to have higher concentrations following oral dosing, but the lung concentrations were markedly higher following intratracheal administration. There was no substantive difference across tissues following dermal dosing, although untreated skin had a slightly higher level; marginal elevations in the liver and kidneys were observed following i.v. dosing. The authors conclude there is wide tissue distribution, with some accumulation in the large organs (lung, liver, and kidney based on within-route comparisons to the levels in blood). Powell et al. (1974) treated juvenile rats (50 g) with ^{14}C-phenol orally or intraperitoneally (i.p.). Based on whole body radiograms, the liver was not a site for accumulation of the phenol; rather, it was widely distributed. It is not clear if the difference between the findings of this study and others is due to the differences in the sensitivity of the analysis.

No direct studies of the placental transfer of phenol were identified. However, Ghantous and Danielsson (1986) evaluated the placental transfer of benzene, of which phenol is a primary metabolite. B6 mice were exposed for 10 minutes to benzene (at a target concentration of 2000 ppm) in air on gestation day (GD) 11, 14, or 17, followed by whole-body radiography analysis and determination of tissue concentrations. Radioactivity was distributed to the fetuses, but not specifically identified as phenol. The concentration of volatile and nonvolatile radioactivity in the fetuses was, however, lower than that in maternal tissues.

The human and laboratory animal data indicate that phenol is widely distributed in the body. Although the human data are inconclusive, the laboratory animal data consistently indicate that highly perfused organs, such as the liver, kidney, and lung have higher tissue concentrations in comparison to the blood concentration.

3.3 Metabolism

Metabolic pathways for phenol are shown in Figure 1. Phenol is directly conjugated with sulfate or glucuronic acid. Phenol that is not directly conjugated can also be a substrate for oxidation reactions. The cytochrome P450 2E1 isozyme (CYP2E1) catalyzes the addition of one oxygen atom to a variety of low molecular weight substrates such as benzene and chloroform, and is thought to be the primary P450 isozyme for phenol oxidation, although a minor role by other cytochrome P450 enzymes cannot be discounted. The oxidation products of phenol generated by CYP2E1 activity appear to be primarily hydroquinone and catechols, which can themselves undergo further oxidation by CYP2E1 to trihydroxybenzene or by peroxidation to benzoquinone. Alternatively, the hydroquinone or catechol metabolites can undergo conjugation reactions. In addition to P450-mediated oxidation, some studies have suggested that peroxidative metabolism of phenol can also take place, producing biphenols and diphenoquinones.

Direct sulfate and glucuronic acid conjugations are detoxifying mechanisms that represent the bulk of phenol metabolism, as evidenced by the metabolic profiles observed in both humans and laboratory animals. In humans and most other species tested, sulfation predominates at the lower doses. Capel et al. (1972) studied the urinary metabolites following oral administration of ^{14}C-phenol to three male volunteers (0.01 mg/kg). In these men, 85-98% of the dose was excreted in 24 hours, 69-90% as phenyl sulfate, 4-23% as phenyl glucuronide, and trace amounts as hydroquinone conjugates. This high degree of conjugation indicates that, at low phenol doses, ingested phenol is nearly completely conjugated.

Laboratory animal studies have clearly shown that as the dose increases, the role of glucuronidation increases, until it becomes the predominant reaction at sufficiently high doses. The formation of oxidative metabolites also increases with increasing dose. These dose-dependent changes have been best characterized in rat studies, which show that at low doses sulfation predominates, with the glucuronidation beginning to predominate at approximately 133 mol/kg (12.5 mg/kg) (Kao et al., 1979; Powell et al., 1974; Hogg et al., 1981; Koster et al., 1981; Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994). There is considerable interspecies variation; however, in the contribution of the sulfation and

glucuronidation pathways (Capel et al., 1972; Mehta et al., 1978).

The formation of oxidative metabolites increases at high doses. Dow Chemical Co. (1994) reported that hydroquinone conjugates represented 3, 7.8, and 17.0% of the eluted radioactivity following single oral doses of 1.5, 15, and 150 mg/kg ^{14}C-phenol, respectively. Legathe et al. (1994) administered an i.p. dose of 75 mg/kg to B6 mice and reported urinary metabolites as 34.5% phenyl sulfate, 28.5% phenyl glucuronide, and 32.4% hydroquinone glucuronide, indicating substantial contribution of oxidative metabolism at this high dose.

The formation of oxidative metabolites is thought to result primarily from reactions catalyzed by CYP2E1. Koop et al. (1989), using hepatic microsomes prepared from male New Zealand white rabbits, showed that CYP2E1 was the most active of six P450 isoforms tested. Treatment of the lysates with an antibody to CYP2E1 inhibited hydroquinone formation by 68% and 89% in acetone-induced and uninduced microsomes, respectively. Snyder et al. (1993) studied phenol metabolism *in vitro* in rat hepatic microsomal preparations. Addition of phenol to the CYP2E1 microsome preparation yielded hydroquinone, and, to a lesser degree, catechol metabolites. Incubation of ^{14}C-phenol and ^{3}H-glutathione in the CYP2E1 microsome preparation yielded an additional metabolite that cochromatographed with the reaction product of benzoquinone plus glutathione. The formation of the glutathione adduct was not dependent on addition of glutathione-S-transferase. Lunte and Kissinger (1983) also reported the formation of glutathione conjugates in microsomal (prepared from liver of male Swiss mice) metabolism of phenol to hydroquinone. In addition to benzoquinone, hydroquinone and catechol can also be oxidized to trihydroxybenzene (Sawahata and Neal, 1983). Additional *in vitro* studies using hepatic microsomes from rats treated with various P450 inducers or inhibitors have also provided evidence for the importance of CYP2E1 in phenol metabolism (Sawahata and Neal, 1983; Gilmour et al., 1986; Chapman et al., 1994; Kenyon et al., 1998). CYP2E1 appears to predominate phenol oxidation. Contributions by other P450 enzymes cannot be excluded; however, since only 68% of the induced hydroquinone formation was blocked by anti-CYP2E1 antibody, and several inducers of other P450 enzymes (such as phenobarbital and arochlor) enhanced phenol metabolism in these studies.

An alternative oxidative pathway involving peroxidation has been described for phenol. Several investigators have used *in vitro* cell preparations with high peroxidase activity, such as peritoneal macrophages or neutrophil preparations (Eastmond et al., 1986; Post et al., 1986; Eastmond et al., 1987; Kalf et al., 1990), purified peroxidase enzymes (Smart and Zannoni, 1984; Subrahmanyam and O'Brien,1985), or cell lines that have high myeloperoxidase activity (Kolachana et al., 1993), to show that phenol can be metabolized in these reactions. Metabolites resulting from these reactions include 4,4'-biphenol and diphenoquinone. While the peroxidation of phenol has been demonstrated *in vitro*, no direct *in vivo* evidence for these peroxidative reactions was identified.

The shift from sulfation to glucuronidation at increasing doses has been postulated to result from depletion of sulfate pools (Kim et al., 1995). Alternatively, it has been suggested that the difference between the Km values for sulfate and glucuronide conjugation drives the conjugation

shift (Weitering et al., 1979). The effects of differing metabolizing enzyme activity across the zones of the liver has also been suggested as an explanation for the metabolic profiles of phenol (Medinsky et al., 1995). The functional units of the liver include lobules with blood supply provided from the perimeter (periportal region) of the lobule though the portal vein and hepatic artery. The blood flows from the periphery of the lobule toward the terminal hepatic vein (also called the central vein) at the center of the lobule through a series of differing metabolic regions or zones. Both sulfotransferases and glucuronosyltransferases are present in periportal zone 1, with the sulfotransferases predominating. Glucuronosyltransferases are present in zone 2, while both glucuronosyltransferases and monooxygenases are present in pericentral zone 3. According to the model, phenol entering the hepatic circulation would be metabolized first in the periportal region where sulfation predominates. Since the blood flows from the periportal region to the pericentral region, and then to the central vein and general circulation, little unconjugated phenol is available for glucuronide conjugation or oxidation when it reaches the pericentral regions of the liver. This model is consistent with the shift from sulfation to glucuronidation at increasing doses. As the dose increases, more of the phenol reaches the pericentral region unconjugated, and thus is available for glucuronidation. The model also explains the increase in oxidative metabolites at high doses that exceed the conjugating capacity of zones 1 and 2 (Kenyon et al., 1995). The model is also consistent with the observation that oral dosage with benzene results in greater production of hydroquinone than that seen after phenol dosage, even though benzene is metabolized to hydroquinone via phenol. Benzene enters the liver in the periportal region, is oxidized in the periportal region, and then leaves the liver via the hepatic vein. Since benzene must be oxidized before it is conjugated, this means that more unconjugated phenol would be released into the blood following benzene exposure than following phenol exposure (Medinsky et al., 1995). Direct evidence for this model was presented by Ballinger et al. (1995), who studied phenol and metabolite transport kinetics in isolated perfused liver from rats, and by Hoffmann et al. (1999), who conducted similar experiments in mice. The effects of enzyme distribution in the zones of the liver were studied by contrasting phenol metabolite profiles resulting from antero- and retrograde perfusions. It is noteworthy that the importance of the location of enzyme activities within the liver would only be significant at oral phenol doses that were not conjugated at the portal of entry, and thus were available for transport to the liver via the hepatic portal vein.

There is some evidence that the capacity for phenol conjugation varies with the portal of entry. Cassidy and Houston (1984) conducted an elegant series of experiments in which phenol was injected intra-arterially, intravenously, or intraduodenally in rats, followed by measurement of the systemic availability of phenol. This allowed the authors to evaluate the first-pass metabolism by different organ systems at doses ranging from 0.4 mg/kg to 15 mg/kg. The authors were able to use this approach to determine metabolism by the liver and gut. However, results from this study on metabolism by the respiratory tract should be treated with caution, since environmental exposure results in exposure of the epithelial respiratory tract (i.e., the portion exposed to the outside), while this study involved exposure of the endothelial respiratory tract (i.e., the portion exposed to the inside of the body). Thus, any differences between the metabolic capacity of the endothelial and epithelial cells would not be taken into account by this study design. In these studies, phenol that was systemically available had not been conjugated or metabolized. The doses at which phenol became systemically available thus reflected the doses at which the relevant

metabolic enzyme systems became saturated. Metabolism became nearly saturated between 4.5 and 15 mg/kg for the endothelial lung, between 0.4 and 1.5 mg/kg in the liver, and was not saturated at the high dose for the gut. The affinity of metabolic pathways also varied among the organ systems. The liver and gut, which removed 88% and 86% (respectively) of phenol at the 0.4 mg/kg dose, demonstrated high affinity compared to the endothelial lung, which removed 58% of the phenol at this same dose. Taken together, these data indicate that the gut is a high-affinity and high-capacity site of metabolism, and the liver has high affinity but its capacity is readily exceeded. The data also suggest that the lung provides substantial metabolizing capacity, but has lower affinity than the gut and liver. Clear conclusions regarding the metabolic capacity of the lung following exposure by the inhalation route are not possible, due to the potential for differences between the metabolic capacity of the epithelial and endothelial cells of the lung. However, one would expect the potential for metabolism of inhaled phenol to be similar to that seen in this study, since systemically absorbed phenol must pass through the endothelial cell layer. The area under the blood concentration curve (AUC) for ^{14}C-phenol was route- and dose-dependent, reflecting the effects of portal-of-entry metabolism. In contrast, the AUC for phenol metabolites did not differ by dosing route, indicating that phenol is extensively metabolized and the effect of portal of entry metabolism is to reduce the amount of parent compound available for metabolism by other organ systems. Studies using isolated perfused rat liver were also conducted and correlated well with the *in vivo* data. The percent of phenol removal from blood by first pass metabolism declined from 73% at a blood concentration of 2.8 g/mL, to 26% at 26 g/mL, indicating extensive saturation at the higher dose level.

Another study that evaluated the differential metabolism kinetics of phenol by differing exposure routes was conducted by Dow Chemical Co. (1994). This study evaluated the kinetics of ^{14}C-phenol in Fischer 344 rats following dosing regimens including single or 8-daily oral gavage doses of 1.5, 15, or 150 mg/kg; 5000 ppm in drinking water for 1 or 8 days; or 25 ppm via inhalation for 6 hours (nose-only) for 1 or 8 days. The authors estimated the doses resulting from the drinking water and inhalation exposures. For drinking water administration (males only), doses were estimated by measurement of daily water consumption. Based on the water intake and the weight of each animal, the administered dose was 291 mg/kg for the single day protocol and 405 mg/kg for the last day of the 8-day treatment; thus the drinking water doses are higher than the oral gavage doses. In contrast, the inhalation doses were estimated as 11.5 and 17.8 mg/kg for males and females, respectively, following a single exposure period, and the dose was 21.4 mg/kg (males only) on the last day of the 8-day exposure protocol. Thus, the inhalation route more closely resembled the mid-dose gavage doses. Metabolic profiles revealed ratios of 0.61 for glucuronide/sulfate conjugates in urine at the two lower gavage doses and were similar following inhalation (0.24-0.39). The ratio at the high gavage dose was 1.16 and was similar following drinking water exposure (1.43 and 1.87 for the single and 8 day exposures). The observed formation of oxidative products, as shown by urine levels of hydroquinone glucuronide, was also dependent on total dose. The formation of oxidative metabolites following inhalation paralleled the low-dose gavage data, while the drinking water levels paralleled the high dose gavage levels. The pattern of phenol metabolism correlated with the magnitude of the absorbed dose and did not appear dependent on the route of administration.

While metabolism of phenol appears extensive in the lung, liver and gastrointestinal tract, limited data are available for other organs. Metabolism appears to be extensive in the kidney (Tremaine et al., 1984). No data were identified that addressed portal of entry metabolism for the skin.

One consequence of the portal-of-entry metabolism of phenol is that phenol serum levels are not necessarily linear with dose or exposure levels. At low doses, almost all of the absorbed phenol is conjugated and excreted, without entering the bloodstream. At higher doses, free phenol and its metabolites appear in the blood and increase with dose. This nonlinearity of blood phenol levels with dose is illustrated by the data of Dow Chemical Co. (1994). Peak phenol blood concentrations in rats following an oral bolus dose of 150 mg/kg were 2320-fold higher than the peak blood concentrations following an oral bolus dose of 1.5 mg/kg.

The role of peak levels may be significant for induction of at least some aspects of systemic toxicity. Dow Chemical Co. (1994) sheds some light on the relationship between metabolism and toxicity. The high-dose gavage group developed a cluster of behaviors that the authors termed "phenol twitching behavior (PTW)," and which included tremors, sudden jerks, hyper-reactivity to stimuli, and excessive blinking. PTW began almost immediately after dosing, and had disappeared by 37 minutes post-dosing. Blood phenol levels also peaked almost immediately after dosing, and PTW was not apparent at blood phenol concentrations below approximately 3 g/mL. PTW was not observed at the lower gavage doses or following inhalation exposure; peak blood phenol levels in these groups were well below 1 g/mL. Interestingly, PTW was also not observed in the drinking-water exposure groups, even though the total dose in these groups was higher than the high gavage dose, and the drinking water doses had a similar metabolic profile to the high gavage dose. Unfortunately, blood phenol levels were not sampled in the drinking water groups, so the peak blood phenol level is not available. However, given the rapid clearance of phenol from the blood, it is likely that the peak blood level was much lower in the drinking water group than the high-dose gavage group. This suggests that PTW is more closely related to peak phenol blood levels than to the area under the AUC of phenol in blood. Because phenol metabolite levels paralleled those of phenol, these data cannot be used to distinguish between phenol and its metabolites being the toxic agent. These data are do not identify the appropriate dose metric (e.g., peak concentration versus AUC) for other toxic endpoints.

One indication that the oxidative metabolites are important determinates of toxicity is based on experiments by Chapman et al. (1994). They studied the dysmorphogenic and embryotoxic effects of benzene and its metabolites to the whole rat conceptus *in vitro*. Phenol at 1.6 mM elicited only minor effects, but inclusion of S9 microsomal fractions greatly increased the potency of phenol, with significant effects observed at doses as low as 0.01 mM. Metabolite analysis indicated that hydroquinone and catechol were the primary metabolites. When evaluated singly, hydroquinone, catechol, and benzoquinone induced similar embryotoxicity, producing 100% lethality at 0.1 mM. The addition of phenol and hydroquinone together induced a more than additive embryotoxicity, which the authors suggest as evidence for a peroxidative mechanism for phenol bioactivation, based on the potential for electron cycling between phenol and

hydroquinone.

Intraspecies variability has also been studied. Campbell et al. (1987) isolated human liver sulfotransferases, the enzymes responsible for the conjugation of phenol with sulfate, and analyzed their apparent activities toward *p*-nitrophenol (as a model compound for simple phenols). The average phenol sulfotransferase (PST) activity measured in liver samples of 20 patients (13 male, seven female) was 35.8±10.6 (SEM) units/mg protein. No correlation between enzyme activity and patient age or gender was found, although the power to detect any such correlation was not noted. Seaton et al. (1995) studied the kinetics of phenol sulfation and hydroquinone conjugation, both of which varied over a range of approximately 3-fold in a sample of liver fractions from ten humans. Using lysates from a single human liver, saturation of phenol sulfation was apparent above 800 M; the observed kinetics were consistent with two contributing enzymes, PST1 and PST2. The expression of two distinct PST enzymes has also been demonstrated in human nasal epithelium (Beckmann et al., 1995).

Kawamoto et al. (1996) studied the effect of various life-style factors and of genetic polymorphisms in five metabolizing enzymes, including aldehyde dehydrogenase (ALDH2), N-acetyl transferase (NAT2), cytochrome P4501A1 (CYP1A1), cytochrome P450 2E1 (CYP2E1), and glutathione-*S*-transferase mu (GSTM1) on urinary levels of phenol in a cohort of men without occupational exposure. Step-wise multiple regression analysis was performed to identify important determinates of urinary phenol levels. Based on this analysis, there was no relationship between polymorphisms (including for CYP2E1) and background urinary phenol levels. In the total sample, (n=351) the geometric mean urinary phenol level was 7.64 mg/L and the GSD was 2.9. No data are available, however, on how genetic polymorphisms affect the levels of metabolites produced from exogenously dosed phenol.

The changes in enzyme activity or expression of genes encoding enzymes important for phenol metabolism with age have been studied. The status of CYP2E1 in fetuses remains unclear, with conflicting results reported. Most of the existing studies indicate that this enzyme is expressed in human adults but not in human fetuses, even when measured using sensitive assays (reviewed in Hakkola et al., 1998). However, at least two studies indicate CYP2E1 is expressed at least to some degree in fetal liver (Carpenter et al., 1996; Vieira et al., 1996). Vieira et al. (1996) found that CYP2E1 protein could not be detected immunochemically in fetal human liver, and there was only minimal evidence of *CYP2E1* mRNA or CYP2E1 activity in fetal liver microsomes. (The difference in assay results may be due to differences in sensitivity, or to cross-reaction of CYP1A1 activity.) The authors found, however, that CYP2E1 protein levels rise rapidly in the first few hours after birth, with a slow increase in protein levels and in *CYP2E1* mRNA levels during childhood. Animal studies of developmental CYP2E1 regulation are consistent with the human data in providing uniform evidence of the rapid induction of this gene soon after birth (Song et al., 1986; Umeno et al., 1988; Schenkman et al., 1989; Ueno and Gonzalez, 1990). Thus, overall the data show that if CYP2E1 activity exists in human fetuses, levels are much lower than those in adults. Regardless of fetal CYP2E1 expression, the enzyme is rapidly induced upon birth. For this reason, children would be expected to be capable of phenol metabolism, although the amount of CYP2E1 may be less than that present in the adult.

Age-dependent changes in phase II conjugation have also been evaluated. In an evaluation of how PST activity varies with age in rats, Iwasaki et al. (1993) studied -naphthol metabolism by PST in fetal rat liver, in the liver of 2-, 9-, 17-, and 25-day-old neonates, and in adult rats. Activity was analyzed in the livers of both sexes. The fetal liver had little conjugating ability, but this activity developed rapidly after birth. However, activity was substantially lower in neonates of all ages evaluated compared to adult levels. Heaton and Renwick (1991) administered 25 mg/kg ^{14}C-phenol i.p. to rats varying in age from 3 to 16 weeks and measured metabolites in urine collected in 24 hours. The percentage of the administered dose recovered in the urine in 24 hours ranged from 61-90% in males and 63 to 99% in females, with increasing recovery with age. Importantly, the formation of hydroquinone conjugates was greater in the younger animals. In males, 38% of the administered dose was recovered as hydroquinone conjugates in the 3-week-old animals and decreased to 8.2% of the urinary metabolites in 16-week-old rats. In females, 17.8% of the administered dose was recovered as hydroquinone conjugates in 4-week-old rats and decreased to 10.5% in 15-week-old rats. Taken together, the evidence indicates that both sulfate conjugation and P450 metabolism are lower early in life and increase as adulthood is reached. However, even in the face of limited P450 activity, significant formation of oxidation products can occur, due to limited sulfation capacity. The oxidative products become substrates for glucuronidation which does not appear to be limited in the young.

Phenol metabolism may also be gender dependent, although the data are less substantial than those for differences due to age. Iwasaki et al., 1986 reported that PST activity was similar in both sexes up to 3 weeks of age and higher in males than females in 7-week old rats; activity in 2-year-old rats of both sexes was similar, and was intermediate between the levels for males and females at 7 weeks to 1 year. Kenyon et al. (1995) administered ^{14}C-phenol to B6 mice of both sexes and observed that at all doses, males excreted a greater proportion of HQ-glucuronide than did females; the difference was roughly 2-fold at a dose of 40 mol/kg. These results are consistent with the greater degree of hydroquinone conjugates excreted in the urine of male versus female rats reported by Heaton and Renwick (1991). Sex-based differences in metabolism have also been reported in rats (Meerman et al., 1987) with slightly lower total recovered radioactivity in the urine of females versus males (i.e. more rapid metabolism in males). However, the magnitude of this difference (91.2% versus 87.3%) was limited.

Interspecies differences in phenol metabolism have also been evaluated. Seaton et al. (1995) found that the rates of both phenol sulfation and hydroquinone conjugation in mouse and rat liver were comparable to those of human liver preparations. Schlosser et al. (1993) reported that mouse liver microsomes metabolized approximately twice as much phenol as did rat liver microsomes, although the relative proportions of metabolites were roughly similar.

Phenol is formed endogenously in the gut by bacterial metabolism of protein. The amount formed is related to the amount of protein ingested, but the amount in humans typically varies from 1 to 10 mg/day, corresponding to approximately 0.014 to 0.14 mg/kg-day (reviewed by Health Canada, 1999).

A physiologically-based pharmacokinetic (PBPK) model for the distribution of benzene

and metabolites was developed by Bois et al. (1991). The model was developed to predict phenol and metabolite distributions to fat, well-perfused tissue, poorly-perfused tissue, bone marrow, liver, lung, and gut, using Monte Carlo simulations of 64 parameters. The model was not validated using empirical data. The Bois et al. (1991) model consistently predicted that phenol administration would produce higher levels of phenol and hydroquinone in the blood than benzene administration.

The first phase of the development of a model of the *in vitro* kinetics of biotransformation of phenol and benzene by liver microsomes was described by Schlosser et al. (1993) and enhanced by Medinsky et al. (1995). The model described the following reaction sequences: benzene > phenol > catechol > trihydroxybenzene; and phenol > hydroquinone > trihydroxybenzene. All reaction steps were assumed to be catalyzed by cytochrome P450 2E1, and benzene, phenol, catechol, and hydroquinone were all assumed to compete through reversible binding for the same reaction site on cytochrome P450. Parameters were identified that were successful at predicting the concentration with time of all five chemicals in incubations with rat or mouse liver microsomes (Schlosser et al., 1993). The observation of a lag time in the production of hydroquinone from benzene, in comparison to the rate of production of hydroquinone from phenol, supported the assumption that all of the substrates compete for the same enzyme reaction site. Medinsky et al. (1995) extended the data into a conceptual model of the differences between phenol and benzene metabolism. Goals of the conceptual model included explaining the observed differences between the carcinogenicity and genotoxicity of phenol and of benzene, and to explain why urinary hydroquinone levels are higher following benzene dosing than after phenol dosing. The latter observation would appear to be inconsistent with the prediction of the Bois et al. (1991) model that blood hydroquinone levels are higher following phenol dosing than following benzene dosing. As described earlier in this section, differences between benzene and phenol toxicity were attributed to zonal differences in the distribution of hepatic metabolic enzymes.

In summary, phenol is an endogenous metabolite that is undergoes further metabolism efficiently. At low doses the bulk of the phenol appears to be conjugated with sulfate or glucuronide at the portal of entry. As the dose increases, the sulfation pathway becomes saturated, and the relative contribution of glucuronidation and oxidation reactions increases. Saturation of first pass metabolism may be important for producing peak levels of phenol that correlate with acute systemic toxicity. In addition, saturation of conjugation, which leads to increases in oxidative metabolism, may also be an important determinate of toxicity. The data on intraspecies variability are limited, but do not indicate great variation in metabolic capacity in humans. In rodents, males and younger animals appear to rely more heavily on oxidative metabolism than females and adult animals, respectively, but the differences are no more than 2-fold. The metabolism of phenol in humans and rodents appears similar, although some evidence suggests that mice metabolize phenol more rapidly than humans or rats.

3.4 Excretion

The existing human and laboratory animal data consistently report that phenol is rapidly excreted, with little tendency for accumulation. Elimination is primarily in the urine in both

humans and laboratory animals, with only a minor contribution of elimination in the bile. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. Workers were exposed to phenol vapor by inhalation on a daily basis. The workers were also possibly exposed by the dermal route, but the contribution of this route to the total exposure was not directly measured. Analysis of urinary phenol levels at different times during the work shift and across work shifts indicated that in workers exposed to 7.8 to 9.6 mg/m^3, the urinary levels increased significantly from the beginning of the work shift to the end of the work shift, but did not tend to accumulate across the work shifts. A slight increase in the morning sample on the sixth consecutive work day was observed, but after two days off, pre-shift samples were no longer elevated. Rapid clearance from the blood has also been observed in humans. Bentur et al. (1998) presented a case report from a dermal poisoning in which a solution of 90% phenol was spilled on the left foot (3% of body surface). Clearance from the blood was rapid, with blood levels decreasing from 21.6 to 2.8 g/mL in the first twelve hours. The authors estimated that the elimination $T_{1/2}$ was 13.86 hours, but they did not include the initial rapid decline in serum concentration that was apparent over the first twelve hours post admission.

Laboratory animal studies have consistently reported that phenol is rapidly excreted. Clearance of phenol from the blood is rapid. Rats given an oral dose of 300 mg/kg had maximum blood concentrations of 26 g/mL at the first measured time point (about 10 minutes), and blood levels declined rapidly to background by 60 minutes (Humphrey et al., 1980). Similar results were observed by the same authors in dogs given a 40 mg/kg dose, with rapid peak levels (7.8 g/mL) and complete removal of free phenol by 1 hour. A $T_{1/2}$ of 12 minutes in blood was reported for rats administered 150 mg/kg by gavage (Dow Chemical Co., 1994). Legathe et al. (1994) reported biphasic elimination kinetics from the blood, with a terminal $T_{1/2}$ of 22 minutes. Similarly, Cassidy and Houston (1984) reported biphasic kinetics with a $T_{1/2}$ of approximately 5 minutes following intra-arterial administration. The elimination kinetics in multiple tissues was studied by Liao and Oehme (1981). Total radioactivity in tissues was maximal within 30 minutes of dosing, representing 28.4% of the administered dose. Tissue levels accounted for 16.6% of the administered dose at 2 hours, and 0.3% at 16 hours. Although maximum levels varied considerably across tissues, the rate of elimination did not appear to differ with tissue type. Based on numerous laboratory animal studies, urinary elimination of sulfate and glucuronide conjugates accounts for most of the excretion, ranging from 70 to 90% of the administered dose within 24 hours, while excretion in feces represents only a small fraction of the administered dose, approximately 1-3% (Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994; Hughes and Hall, 1995).

4. HAZARD IDENTIFICATION

4.1 Studies in Humans - Epidemiology, Case Reports, Clinical Controls

The epidemiology data on phenol are limited. These studies typically included confounding exposures and did not adequately adjust for smoking. Kauppinen et al. (1986) reported a significant increase in respiratory cancer in phenol-exposed workers, but this observation appears to be due to confounding exposures, since there was no dose-response and the

effect decreased after accounting for latency. No effect on cancer mortality was observed on workers exposed to phenol in the rubber industry (Wilcosky et al., 1984) or in workers exposed to formaldehyde and phenol (Dosemeci et al., 1991). An occupational study (Shamy et al., 1994) and case studies (e.g., Merliss, 1972) reported liver effects following exposure to phenol. Immune effects have also been reported in an occupational study of workers exposed to phenol as part of a mixture of solvents (Baj et al., 1994). Studies of populations drinking water contaminated with phenol reported elevated incidences of diarrhea, nausea, mouth sores, and dark urine (Jarvis et al., 1985; Baker et al., 1978).

4.1.1 Oral

Jarvis et al. (1985) reported a retrospective mail survey of 594 English households on illness associated with an incident of contamination of drinking water supplies with phenol. The river that served as a drinking water source for the exposed households was contaminated by a spill of phenol into the river. The survey was sent to 250 unexposed households selected from the telephone book, 94 households exposed to low concentrations (from a reservoir that diluted the contaminated river), and 250 highly-exposed households. Based on the data from the water authority, the estimated phenol concentrations in the low-exposure area (0.05 g/L, equivalent to 4.7 g/L) was roughly half that in the high-exposure area (0.11 g/L, equivalent to 10 g/L) for the first 24 hours. The next day, the phenol concentration for both groups was 0.05 g/L, and the concentration was <0.01 g/L (<0.9 g/L) by the third day after the contamination incident. Chlorination of the water resulted in production of chlorophenols. The chlorophenol concentration followed a similar pattern, but the chlorophenol concentrations, which ranged from 0.43-0.2 g/l at the first measurement (assuming all chlorophenols were in the form of trichlorophenols), were higher than that for phenol. There were no data on phenol concentrations in the unexposed area, but an unspecified local press report implicated a possibility of phenol contamination. Due to the similarity between the two exposed areas in the measured concentrations of phenol and chlorophenol, these two areas were combined in the data analysis.

The percentage of responding households was similar in all of the groups, and ranged from 69% to 77%. This resulted in 172 households (448 people) in the unexposed area, and 254 households (754 people) in the exposed area being evaluated. The two groups had similar distributions in sex, age, and usual water usage. Compared to the unexposed people, the people in the exposed area had significantly higher incidences of gastrointestinal illnesses, such as diarrhea, nausea, vomiting, and abdominal pain. Other symptoms, such as headache, rash, and malaise, were also observed at a significantly elevated incidence in the exposed group. The day of onset of symptoms corresponded with the period of elevated phenol concentrations in the contaminated drinking water. The associations were stronger among those reporting that they drank the water than among those in the exposed area who reported not drinking the water. (Others may have consumed the water in cooking.) In another analysis, gastrointestinal symptoms did not significantly correlate with whether the water tasted bad. However, because of the confounding exposure of chlorophenol in the water, the use of the result for risk assessment is limited.

Baker et al. (1978) reported phenol poisoning in humans due to an accidental

contamination of their drinking water on July 16, 1974. A train derailment resulted in a spillage of 37,900 liters of pure phenol on the ground, and the spillage caused contamination of drinking water in wells of nearby houses. Two wells near the spill were tested initially on July 23, and were found to have phenol concentrations of 0.21 and 3.2 mg/L. Further testing in late July and August of the six wells nearest the spill found peak concentrations between 15 and 126 mg/L. Within approximately two months after the spill, "most families" began to obtain water from other sources (from neighbors or bottled water). Phenol concentrations in well water (unspecified number of wells tested) as high as 1130 mg/L were reported over the next 6 months, with the higher levels observed after flushing of the spill site. The authors investigated the health effects in three groups of people. Group 1 (n=39) consisted of all those living 120-310 meters from the spill site and having at least one water test greater than 0.1 mg phenol/L (at least once between July and February). Group 2 (neighborhood control) (n=61) consisted of all families adjacent to Group 1 (210-670 meters from the spill) whose wells had phenol concentrations of between 0.1 to 0.0001 mg/L. Group 3 (distant control) (n=58) lived at least 1.9 km from the spill and had no phenol in their wells.

Group 1 reported significantly more diarrhea, mouth sores, burning mouth, and dark urine than in the combined control groups. About 44% of Group 1 reported at least two of these responses in 7 months following the accident, and were considered "affected individuals"; only 8% and 3% of Groups 2 or 3 subjects, respectively, had the same responses. Responses in Group 1 were primarily restricted to the first two months of exposure, before the use of bottled water began. Responses in the other two groups tended to occur throughout the eight-month period (July through February). Other than the four reported symptoms, no abnormal observations in physical examinations and serum biochemical evaluations were evident in Group 1 compared to controls when monitoring was done in February.

Based on water testing data and water preference histories, the authors estimated that the daily oral dose of phenol for the 17 affected individuals in Group 1 was between 10 and 240 mg. This range may overestimate the amount of phenol ingested, however, since phenol's unpleasant odor might discourage ingestion of water with concentrations above 0.1 mg/L. In contrast, this range does not include phenol that may have been absorbed during skin contact with contaminated water. It was also not clear whether the subjects continued to shower with the contaminated water after switching their drinking water source. Based on a default adult body weight of 70 kg, this daily oral dose corresponds to 0.14 to 3.4 mg phenol/kg-day. Thus, there is a considerable range in the estimated phenol dose associated with symptoms. In addition, since "most" (but apparently not all) families switched to other water sources within the first two months of exposure, the exposure duration for the affected individuals is not known. Therefore, it is difficult to use these data for quantitative analysis, although this information might be useful to place bounding estimates on the risk values estimated from laboratory animal studies.

4.1.2 Inhalation

Kauppinen et al. (1986) reported a case-control study on respiratory cancers and chemical exposures in the wood industry. A cohort of 3805 Finnish men who worked in the particle board,

plywood, sawmill, or formaldehyde glue industries for at least one year between 1944 and 1965 was followed until 1981. From the cohort, 60 cases of respiratory malignant tumors were identified. The tissue locations of these tumors included tongue (one), pharynx (one), larynx or epiglottis (four), and lung or trachea (54). No cases with tumor in the mouth, nose, or sinuses were identified. Among the 60 cases, two were rejected due to a false preliminary diagnosis of cancer and one was rejected as having chronic lymphocytic leukemia. The final size of the group of cases was thus 57. The control group contained three subjects for each case, selected from the cohort and matched by birth year; thus, the total size of the control group was 171. The job exposure was estimated based on the industrial hygiene data of the plant, general hygiene data on exposures, and information on ventilation, work procedures, and other relevant factors at the plants. The study authors, however, did not mention any information on direct phenol measurements; thus, the quality of the estimated exposure levels could not be evaluated. The work histories of the subjects were assessed primarily based on plant registers supplemented with personal interviews, and the individual phenol exposures were determined qualitatively as yes or no, and as a function of exposure time. Smoking histories were determined by a mail survey that provided smoking information on 39 of 57 cases (68%) and on 130 of 171 controls (76%). Because there were few non-smokers and information on the amount smoked was not as complete as on years of smoking, the subjects were only divided as to light or heavy smokers, based on whether their years of smoking exceeded 35 years.

Phenol exposure resulted in a statistically significant odds ratio (OR) of 3.98 or 4.94 for respiratory tumors with or without the adjustment for smoking years, respectively. When the duration of phenol exposure was considered, both exposures 5 years and > 5 years resulted in a statistically significant OR of 5.86 or 4.03, respectively (i.e., no duration response). When a provision for a 10-year latency was introduced (excluding exposure during the 10 years immediately preceding the diagnosis of cases), phenol exposure resulted in a non-significant OR of 2.86 adjusted for smoking years, but a significant odds ratio of 3.98 without smoking adjustment. Of the 39 cases for which smoking information was available, 12 had been exposed to phenol (9 to phenol in wood dust), and 7 had been exposed to phenol with a 10-year latency (4 to phenol in wood dust). Since the OR did not increase with duration of phenol exposure, and the provision for the 10-year latency period resulted in lower values of ORs, a confounding factor may have been responsible for the observed statistically significant ORs.

One of the confounding factors could be concurrent exposure to multiple pesticides, which was shown to increase the OR for respiratory tumors in the same study. An exclusion of workers exposed to both phenol and pesticides resulted in a change of the OR from a significant 4.9 to a non-significant 2.6. Thus, a confounding effect due to exposures to pesticides was very possible. Based on the location of the tumors, formaldehyde exposure is also a likely confounder.

Generally similar results were observed in this study with workers exposed to phenol in wood dust. Exposure to phenol in wood dust resulted in a statistically significant OR with or without adjustment for smoking. As for the workers exposed to phenol but not wood dust, provision for a latency period eliminated the observed statistically significant OR. Among the workers exposed to phenol in wood dust, however, the OR did increase with exposure duration,

and was statistically significant in those exposed >5 years (OR of 4.77), but not in those exposed for 5 years (OR of 3.84). Based on these results, the phenol-exposed workers had an elevated risk of respiratory cancer, but phenol itself does not appear to be the causative agent; rather, it appears that there was a confounding exposure.

Wilcosky et al. (1984) reported a case-control study of cancer mortality and solvent exposures in the rubber industry. From a cohort of 6678 active and retired male rubber workers of a large plant in Ohio, 183 decedents from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, and respiratory cancer were selected as cases. As a control, 20% of an age-stratified random sample of the cohort (calculated as 1336 subjects) were selected. Including phenol, a total of 25 solvents were authorized to be used in the plant. The exposure to any particular solvent was determined based on the records of annual authorization for use of these solvents in each work area. Only workers with cumulative exposures of more than one year were considered exposed.

Based on the analysis of the age-adjusted exposure ORs, no association was seen between phenol exposure and mortality from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, or respiratory cancer. However, this study had several major limitations. One limitation was that the estimation of whether workers were exposed to a solvent was solely based on authorization and not actual usage. (This limitation would tend to lead to an overestimation of exposure.) In addition, the analysis was based solely on a qualitative evaluation of whether a given solvent was used; no estimates of exposure were made, and so no exposure-response assessment was conducted. Although smoking can confound evaluation of cancer risk, this factor was not investigated. Finally, it was common for workers to be simultaneously exposed to multiple solvents; therefore, solvents other than phenol may have also affected the study outcome. In this study, phenol exposure was not associated with a risk of several cancers, but this lack of an association cannot be considered definitive because of study limitations mentioned above.

In an occupational epidemiology study, Dosemeci et al. (1991) evaluated mortality among 14,861 white male workers in five companies that used formaldehyde and phenol. Unfortunately, the phenol exposure was confounded by co-exposure to other compounds, such as formaldehyde, asbestos, urea, melamine, hexamethylenediamine, wood dust, plasticizers, carbon black, ammonia, and antioxidants. Based on phenol concentrations obtained from historical monitoring and industrial hygiene surveys, the authors assigned each job/department/year combination to groups with no, low, medium, or high phenol exposure, and calculated cumulative exposure. Among the entire cohort, there were no significant increases in standardized mortality ratios (SMRs), compared with the entire U.S. population, for all causes of death or any diseases. The phenol exposed workers as a group had slightly elevated SMRs for cancers of the esophagus (1.6), rectum (1.4), kidney (1.3), and Hodgkin's disease (1.7); however, none of these increases were statistically significant compared to the general population. In addition, analysis of mortality by level of cumulative exposure showed that none of these increases had dose-response relationships with exposure to phenol. The only significant observations were decreases of SMRs for infective and parasitic diseases, and for accidents in the entire cohort and exposed workers. These

observations were attributed to the healthy worker effect. This study provided no evidence of phenol-induced morbidity, mortality, or carcinogenicity.

Baj et al. (1994) reported an epidemiology study of 22 Polish office workers (18 females and 4 males) exposed to Ksylamit® vapor for 6 months and 27 age- and sex-matched healthy volunteers from the same town. The exact composition of the Ksylamit® vapor was not reported. The study authors stated that Ksylamit® consists of "a mixture of chlorinated benzenes, pentachlorophenol, -chloronaphthalene, chloroparaffin and kerosene." The only exposure information reported was that at the end of 6-month exposure, the concentrations of formaldehyde and phenol in the workplace atmosphere were 0.8 mg/m^3 and 1.3 mg/m^3, respectively. The study authors did not address how exposure to formaldehyde or phenol resulted from the reported product constituents. In addition, it cannot be determined from the presented information whether the analytical methods used would differentiate between phenol and pentachlorophenol (ATSDR, 1998). The exposed workers reported chronic symptoms such as headache, cough and sore throats, burning eyes, and fatigue, but the morbidity during the 6-month exposure period was comparable to that of the controls. Although all evaluated hematological parameters were normal in the exposed workers as a group, some statistically significant changes were observed in a subset of eight workers who had elevated urinary phenol levels 3 days after the last day of exposure (mean of 18.2 mg/L, compared with 12.1 mg/L in the exposed workers, and 7.9 mg/L for the general population). Compared with the matched controls, there was a small, but statistically significant decrease in erythrocyte counts, and a statistically significant *increase* in eosinophil and monocyte counts. Levels of CD3, CD4, and CD8 lymphocytes were also decreased in the exposed group, but there was no effect on the CD4/CD8 ratio, and the effect was not stronger in the apparently more highly-exposed subset. Decreases in lymphocyte proliferation induced by phytohemagglutinin (PHA) and alloantigens were also observed in exposed workers, while reactivity to concanavalin A (Con A) was unchanged. These results suggest that exposure to Ksymamit® could affect the immune and hematological systems. However, the poor characterization of the chemical exposure, including uncertainties regarding the source of the phenol, as well as the marginal dose-response for phenol in urine, mean that conclusions regarding the contribution of phenol to the observed effects are limited.

Shamy et al. (1994) reported a cross-sectional investigation of phenol-induced biochemical changes in workers at an oil-refining plant in Egypt. The study subjects included 20 subjects were exposed to a TWA concentration of 5.4 ppm phenol and 30 office workers with no exposure to organic solvents. The phenol-exposed workers worked in the aromatic extraction of distillates; other potential exposures were not described. The mean concentration of phenol in spot urine samples was 68.6 and 11.5 mg/g creatinine in the exposed and control groups, respectively. The average duration of exposure was 13.15 years. At the end of the shift of the last working day of the week, blood samples were collected for hematological and serum biochemistry evaluations. Small but statistically significant increases (approximately 55% and 80%, respectively) were observed in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). There were also small but statistically significant *increases* in hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), although there was no effect on red blood cell count. This is in contrast with the

findings of laboratory animal studies, in which *decreases* in erythrocytes and hematocrit have been reported. Other small, but statistically significant changes included increased basophils and neutrophils, decreased monocytes, and increased clotting time. A nonsignificant increase in prothrombin time and decrease in platelets was also observed. Overall, these data suggest subclinical effects on the liver and hematopoietic system, based on the small changes in SGOT and SGPT; the observed increases in the hematology endpoints are not adverse. Although the study authors described the group as exposed to phenol alone, and compared them with other workers exposed to mixed solvents, it appears that the phenol-exposed workers may have also been exposed to other organic compounds which can cause hepatic or hematologic effects, and the observed effects cannot be clearly attributed to phenol exposure.

Merliss (1972) reported a case of phenol marasmus. A laboratory technician had been frequently exposed to phenol vapor or through skin contact for 13.5 years. He developed lessened appetite, weight loss, muscle pain in his legs and arms, and dark color in his urine. Serum biochemical evaluations indicated liver damage, with SGOT and SGPT levels much higher than the normal levels. The patient's symptoms improved after the exposure ended.

4.2 Pre-chronic, Chronic Studies and Cancer Bioassays in Laboratory Animals

4.2.1 Oral

Estimated lethal oral doses in adults vary widely, from as much of 1 g (14 mg/kg assuming adult body weight of 70 kg) to 65 g (930 mg/kg) (Deichmann and Klepinger, 1981). In a separate report, the minimum lethal oral dose in adults was estimated as 140 mg/kg (Bruce et al., 1987).

Acute lethality of oral phenol has been evaluated in numerous animal studies. Oral LD_{50} values in rodents range from 300 mg/kg to 650 mg/kg (Deichmann and Witherup, 1944; Von Oettingen and Sharpless, 1946; Flickinger, 1976; Berman et al., 1995). The acute toxicity of phenol when administered by gavage appears to be at least partly dependent on phenol concentration or total administered volume (Deichmann and Witherup, 1944; NTP, 1983a) and might be more severe in young animals (Deichmann and Witherup, 1944). In addition to lethality, acute oral dosing has been reported to induce adverse renal (tubular necrosis, protein casts, papillary hemorrhage), hematological (reduction in poly/normochromatic erythrocyte ratio), respiratory (dyspnea and rales), neurological (muscle twitching, decreased motor activity, ataxia, tremors, convulsions, coma) and reproductive and developmental effects (Berman et al., 1995; Narotsky and Kavlock, 1995; Liao and Oehme, 1981; Moser et. al. 1995; NTP, 1983a, 1983b).

An extensive database of oral studies relevant to the RfD exists, as shown in Table 2. Chronic drinking water studies were conducted in rats and mice, but the only noncancer endpoints evaluated were body weight and histopathology (NCI, 1980). Hematology and serum biochemical evaluations were included in a recent 2-generation drinking water study conducted in rats (IIT Research Institute, 1999). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch Ltd., 1998). These drinking water studies consistently found effects only at exposure levels where water consumption was also decreased, sometimes by as much as 80%. The decreased water consumption was presumably due

to poor palatability of the drinking water. Effects seen in the drinking water studies included tremors, decreased final body weight compared to the controls (possibly as a result of dehydration), decreased motor activity, and kidney inflammation. Decreased pup survival was also observed in the 2-generation study. The only drinking water study in which effects were seen in the absence of decreased water consumption was a 28-day study with mice by Hsieh et al. (1992). In that study, decreased hematocrit and decreased immune endpoints were observed at doses at least an order of magnitude lower than the NOAELs in the other drinking water studies. As described below, there are a number of uncertainties and limitations associated with the Hsieh et al. (1992) study. It is unclear if the difference between this study and the 2-generation study in rats (IIT Research Institute, 1999), in which no hematological effects were observed, is due to differences between rats and mice, or to uncertainties in the Hsieh et al. (1992) study. However, as discussed in more detail later in this document, other toxicity data do not indicate such large differences between rats and mice.

Toxicity in gavage studies with phenol is typically much higher than that in the drinking water studies. NOAELs for systemic effects were 5-10 fold lower in gavage studies (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945) than those seen in the drinking water studies. Effects observed included tremor, and liver and kidney histopathology. As described in greater detail in Section 4.5, this difference between gavage and drinking water exposure is consistent with toxicokinetic data suggesting that toxicity is correlated with peak blood concentrations, rather than the area under the curve (AUC). Several developmental toxicity studies are available (Argus Research Laboratories, 1997; NTP, 1983a; NTP, 1983b, Narotsky and Kavlock, 1995) all via the gavage route, although the Argus Research Laboratories (1997) study used large dosing volumes and a divided dosing protocol, apparently to reduce the effect of peak blood levels. The developmental toxicity studies found that the primary fetal effect is decreased fetal body weight, which occurs at doses similar to those that cause decreased maternal body weight gain. NTP (1983a) also found that toxicity increased markedly if the same phenol dose was administered in a lower dosing volume. Since the observed signs of toxicity included tremors and liver and kidney pathology, and were not targeted to the portal of entry, the effect of dosing volume is not related to the concentration of a direct-contact toxicant.

NCI (1980) conducted a carcinogenicity bioassay in which F344 rats (50/sex/group) received analytical grade phenol (approximately 98.5% pure) in drinking water at concentrations of 0, 2500, or 5000 ppm for 103 weeks, and were sacrificed 1-2 weeks later. Using the reference water intake of 0.13 and 0.14 L/kg-day for chronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 260, and 585 mg/kg-day for male rats; and 0, 280, and 630 mg/kg-day for female rats. The doses shown here were adjusted to account for the reported water consumption of 80% and 90% of control at the low and high doses, respectively. The animals were observed daily for clinical signs and examined weekly for palpable masses. Body weights and food consumption were recorded every 2 weeks for the first 12 weeks and then monthly thereafter; water consumption was recorded weekly. At the end of study, the animals were killed, and complete gross and histopathological examinations were performed. Organs and tissues examined included the bone marrow, spleen, cervical and mesenteric lymph nodes, heart, liver, kidney, thyroid, reproductive organs, brain, and other major

tissues. No evaluation of other noncancer endpoints, such as hematological effects or serum biochemistry, was conducted. The survival rate at study termination was comparable among all three groups of males (approximately 50%) and females (approximately 75%). Dose-related decreases in body weight compared to the controls were observed in male and female rats, with a decrease of approximately 15% in high-dose males and approximately 10% in high-dose females. Water consumption was reduced by approximately 10% at the high dose. The study authors stated that the non-neoplastic lesions were similar to those naturally occurring in aged F344 rats. However, an analysis conducted for this assessment found statistically significant increases (using a Chi square test) in chronic kidney inflammation in high-dose males and females; there were no significant changes at the low dose. No other differences in the incidence of non-neoplastic lesions between the controls and exposed rats were observed. Based on increased kidney inflammation and decreased body weight compared to controls at the high dose of 5000 ppm (585 mg/kg-day for males and 630 mg/kg-day for females), the NOAEL in this study can be considered to be the low dose, 260 mg/kg-day in males and 280 mg/kg-day for females, resulting in an overall study NOAEL of 260 mg/kg-day. These effects also indicate that the MTD was reached.

In the NCI (1980) rat study, there were no dose-related trends in cancer incidence in male or female rats, but the study authors reported several tumors for which statistically significant increases were seen in low-dose males only, based on pairwise comparisons. These increases were seen in the incidences of pheochromocytomas of the adrenal medulla (13/50, 22/50 and 9/50 in the control, low-, and high-dose groups, respectively), and "leukemias or lymphomas" (18/50, 31/50 and 25/50). The historical control incidences of pheochromocytomas in the bioassay program was 9% (data for the test laboratory were not reported), and the historical control incidence of leukemias or lymphomas in the test laboratory was 26%. The study authors stated that the leukemias were "of the type usually seen in untreated F344 rats." There were no significant increases in tumor incidence in any tissue in female rats. Because there was no clear dose-response in males and the tumors were not observed in female rats, an association between the tumors and phenol exposure cannot be established. The NCI concluded that phenol was "not carcinogenic in male or female F344 rats." However, the report noted uncertainties regarding the possible increase in leukemia in male rats, and the NCI reviewers recommended that phenol be considered for a retest.

In a parallel study, NCI (1980) administered phenol at 0, 2500, or 5000 ppm in drinking water to B6C3F1 mice (50/sex/group) for 103 weeks, and sacrificed the mice 1-2 weeks later. For B6C3F1 mice, the reference water intake is 0.24 L/kg-day for both sexes. The study reported that water consumption was decreased to 75% and 50-60% of the control levels at the low and high doses, respectively. The resulting doses (adjusting for decreased water intake) are 0, 450, and 660 mg/kg-day for both sexes. Dose-related decreases in body weight compared to the controls were attributed to the decrease in water consumption. No other clinical signs of toxicity were observed, and mortality rates (approximately 10% in males and 20% in females) were comparable between experimental and control groups. Histopathological examination and statistical analyses revealed no phenol-related signs of toxicity or carcinogenicity; lesions in all systems observed in the dosed groups were comparable to those in the controls. NCI concluded that, under the conditions of the assay, phenol was not carcinogenic in male or female B6C3F1

mice (NCI, 1980). Based on the decreased body weight compared to controls observed at 5000 ppm, the low dose of 2500 ppm (450 mg/kg-day) can be considered the study NOAEL. The observed effect, however, is likely secondary to the decreased water consumption due to poor palatability.

In light of the marked decrease in water consumption, higher doses of phenol in drinking water probably could not have been tested. If the study authors attempted to overcome the palatability issue by administering the high dose in the NCI (1980) mouse study by gavage, instead of in drinking water, high toxicity would have been expected, in light of the higher toxicity of phenol administered by gavage compared to phenol in drinking water (see Section 4.5 and Table 2). These considerations suggest that an MTD was also reached in mice, although the conclusion is less clear than for rats.

In the range-finding test for the carcinogenicity bioassay (NCI, 1980), F344 rats and B6C3F1 mice (10/sex/group) were given drinking water containing 0, 100, 300, 1000, 3000, or 10,000 ppm phenol (98.47% pure) for 13 weeks. Using the reference water intake of 0.16 and 0.17 L/kg-day for subchronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 16, 48, 160, 480 and 800 mg/kg-day for male rats; and 0, 17, 51, 170, 510, and 1140 mg/kg-day for female rats. The high doses shown here were adjusted to account for the decreased water consumption described below. For B6C3F1 mice, the reference water intake is 0.25 L/kg-day for males and 0.26 L/kg-day for females. The corresponding doses (adjusting for decreased water intake at the high dose) are 0, 25, 75, 250, 450, and 500 mg/kg-day for males; and 0, 26, 78, 260, 468, and 520 mg/kg-day for female mice. Body weights, appearance, behavior, and food and water consumption were recorded weekly. After 13 weeks, all animals were killed and tissues were subjected to histopathological examinations. All of the rats and mice survived the phenol treatment. The only significant observation was the decreased final body weights (compared to controls) in rats of both sexes (11%-14%) and male mice (12%) that received 10,000 ppm. Since drinking water consumption in these groups was decreased to 50-70% (rats) and 20-60% (mice) of the control value, the decreased body weight was likely due to the low water consumption. No histopathological changes attributable to phenol treatment were observed. This study suggests that the second highest dose (480 mg/kg-day in male rats, 510 mg/kg-day for female rats, 450 mg/kg-day for male mice, and 470 mg/kg-day for female mice) was a NOAEL, based on the decreased final body weight (compared to controls) at 10,000 ppm, which was secondary to decreased water consumption due to poor palatability at the high dose.

In an unpublished 13-week neurotoxicity study conducted according to GLP guidelines (ClinTrials BioResearch Ltd., 1998), groups of 15 male and 15 female Sprague-Dawley rats received phenol via drinking water at concentrations of 0, 200, 1000, or 5000 ppm phenol (100% pure) for 13 weeks, followed by a 4-week recovery period.[2] The study authors calculated that the

[2]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for a neurotoxicity screening battery), is well-documented, and contributes useful

average doses were 0, 18.1, 83.1 and 308.2 mg/kg-day for males and 0, 24.6, 107.0 and 359.8 mg/kg-day for females. These concentrations in the drinking water were chosen based on preliminary palatability studies conducted at a different laboratory (IITRI project No. L08657).[3] During the exposure period, clinical signs and water intake were recorded daily, and body weight and food consumption were recorded weekly. In addition, a functional observational battery (FOB) and a motor activity test were conducted pre-study and once during each of weeks 4, 8, 13, and 17. At the end of the exposure and the end of the recovery period, 5 rats/sex in the control and 5000-ppm groups underwent neuropathological evaluations (including a thorough evaluation of the brain and several nerves). The rest of the rats in the ClinTrials BioResearch Ltd. (1988) study were killed at the end of the 4-week recovery and were subjected to gross necropsy.

One high-dose female was euthanized on day 14 due to poor condition. Clinical signs of this female prior to sacrifice included dehydration, hunched posture, tremors, reduced activity, and cold to touch. Among the rest of the high-dose animals, the primary clinical sign was dehydration, which was accompanied by reduced activity and tremors in one female, and by a thin appearance in additional animals. Dehydration was also observed in mid-dose rats (2/15 in each sex). Dehydration was assessed qualitatively, and independently of drinking water consumption, by grabbing the scruff on the back of the animal's neck; a delay in returning to the normal position was considered dehydration (P. Beyrouty, personal communication). The dehydration was associated with marked decreases in water consumption at the high dose, and smaller decreases at the mid-dose. Decreases in water consumption were more pronounced in females than in males and were most evident during the first week of dosing. Water consumption was decreased to approximately 90% of the control level in mid-dose males and females, approximately 60% of control levels in high-dose males, and to approximately 55% (40% during the first week) of control levels in high-dose females. Water consumption rebounded to levels higher than controls during the recovery period. The decreased water consumption was likely due to the poor palatability of phenol at high concentrations, rather than a manifestation of an overt toxicological effect. In addition, the high-dose group had decreased body weights compared to the controls (8% for males and 12% for females) and decreased food intake (approximately 10% for males and 10-20% for females).

The only toxicologically-significant neurological effect was decreased motor activity in females. There was a statistically significant reduction in total group mean motor activity counts at week 4 in the 5000-ppm group. The authors reported that the rate of linear change of motor activity with time was also significantly decreased at weeks 8 and 13 in the 1000- and 5000-ppm groups, although supporting data were not provided. Motor activity in females at week 4 exhibited

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information to the hazard identification and dose-response portions of the assessment. The study was designed to comply with the U.S. EPA Enforceable Consent Agreement for Phenol (Docket No. OPPTS-42150).

[3]Results of the palatability study were not provided in the IITRI study report (IIT Research Institute, 1999), which reports the results of the 2-generation reproduction study. The reproduction study was conducted during the same time period as the neurotoxicity study, and reported similar problems of markedly decreased drinking water consumption at the high dose of 5000 ppm (see Section 4.3).

a dose dependency at the first five (of six) analysis intervals, although the total counts for the low- and mid-dose groups were not significantly different from control. High-dose females also had markedly lower total activity counts than controls, and lower counts in the first four intervals, at week 4, although there was no statistically significant difference in mean total counts (Table 3). By contrast, the high-dose males had markedly lower group mean activity counts prestudy, but activity comparable to, or higher than, the controls at weeks 4, 8, and 13.

The study authors attributed the decreased activity to dehydration, noting that the control group mean total activity increased by >20% at week 4 compared to prestudy levels, while activity of dehydrated females in the 5000- ppm group at week 4 was decreased by 17% and activity of females in this group that were not dehydrated increased by 2%. To address whether the decreased activity could be attributed to dehydration, this assessment evaluated the data in greater detail. Table 4 presents the individual animal data for week 4 total motor activity counts and compares it with the individual animal dehydration data. If the individual clinical data reported an animal as dehydrated, the days of that notation are shown. With the exception of animal 4502 (which died) and animal 4507, which had severe dehydration, dehydration was noted as slight or moderate. For clarity of presentation, the individual animal data are shown for the control and high-dose groups, while only the average data are shown for the low and mid-dose groups. Only two animals in the mid-dose group were reported as dehydrated on any day, and neither of these animals had decreased motor activity. As shown, the average activity was lower in the dehydrated high-dose females than in those not reported as dehydrated, but an association of decreased activity with dehydration was not consistently supported on an individual animal basis. (For the purpose of calculating averages, animals were considered dehydrated if they dehydrated at any point in the study. This is a limitation to the analysis, since some were reported as dehydrated only prior to week 4, and others were reported as dehydrated only after week 4. In addition, basing the analysis on the clinical sign of dehydration may not appropriately reflect whether the animals were dehydrated, since no objective measure of dehydration was used, and since decreased water consumption in this group occurred throughout the study.) As shown in Table 4, animal #4601 was reported as dehydrated on days 14 and 21, but had one of the highest total activity counts. Conversely, animal #4512 had the lowest activity count, but was never reported as being dehydrated. Furthermore, the mean activity of the dehydrated high-dose females was 67% of concurrent controls, compared to 78% of concurrent controls for the non-dehydrated high-dose females. These data indicate that the difference between the control and high-dose animals was greater than the difference between the dehydrated and non-dehydrated animals at the high dose. Overall, the data indicate that there is not a tight linkage between dehydration and decreased motor activity in the high dose females. The data for high-dose males also did not indicate a clear correlation between low activity and dehydration. The clinical signs for one high-dose male (#4003) for week 2 included severe dehydration and decreased activity, but no effect (of dehydration or decreased activity) was seen when the animal underwent the objective activity analysis in week 4. The finding of dehydration in males without the accompanying decrease in activity further supports the conclusion that only severe dehydration (not mild or moderate) results in decreased motor activity levels, and the decrease observed in females was phenol-related. Conversely, the absence of other findings in the FOB, and the presence of a statistically significant effect on motor activity only at 4 weeks, and not at later time points, argue against a neurotoxic

effect of phenol.

As an additional investigation of whether the decreased motor activity was related to dehydration, the literature on water deprivation and motor activity were reviewed. This literature is very limited. However, Campbell and Cicala (1962) evaluated the effects of terminal water deprivation and of terminal food deprivation (i.e., deprivation until death from starvation or dehydration) on motor activity of male and female Wistar rats. Motor activity was measured using a stabilimeter, which is similar to the figure 8 mazes used in the ClinTrials BioResearch Ltd. (1998) study, in that ambulation (as opposed to simply movement) is measured. Campbell and Cicala (1962) found that water deprivation alone did not result in decreased motor activity until approximately day 5-7 (depending on age), at which time activity continuously declined until death. By contrast, food deprivation resulted in an initial increase in activity, followed by decreasing activity until death. Only the pooled data for males and females together were reported. These results are not directly comparable to the results of the ClinTrials study, since the latter involved long-term, lower-level dehydration. However, the results from the Campbell and Cicala (1962) do support the conclusion above that the decreased motor activity in high-dose females was due at least partially to phenol exposure. The most appropriate way to address this issue would be to conduct the neurotoxicity study with a water-restricted control group. Overall, based on the decreased motor activity, the study NOAEL in females was 1000 ppm phenol (107 mg/kg-day) and the LOAEL was 5000 ppm (360 mg/kg-day). No LOAEL was identified in males; the high dose of 308 mg/kg-day was a NOAEL.

Hsieh et al. (1992) investigated the effects of phenol exposure on hematological, immune, and neurochemical endpoints in a study of 6-week-old male CD-1 mice administered actual concentrations of 0, 4.7, 19.5 or 95.2 ppm in drinking water for 28 days. Based on measured concentrations and "estimated" water intake, the study authors reported that the corresponding daily doses were 0, 1.8, 6.3, and 33.6 mg/kg-day. However, the accuracy of the dose estimate may be questionable. The water concentrations and corresponding doses reported by the authors correspond to a water intake of 0.31-0.38 L/kg-day, which is much higher than the EPA reference values of approximately (depending on the strain) 0.25 L/kg-day (U.S. EPA, 1988). It is also unclear how accurately water intake was measured, since the study authors reported that water consumption was monitored throughout the study, but the doses were calculated based on estimated water intake. If the EPA reference values of 0.25 L/kg-day and the reported concentrations are used to calculate the dose, the actual doses would have been 0, 1.2, 4.9, and 24 mg/kg-day.

The mice were housed in groups of five/cage. Drinking water was prepared and changed every 3 days. Drinking water was provided in glass water bottles with stainless sipper tubes containing ball bearings, to minimize evaporation; the bottles were shaken frequently during treatment. Food and water consumption were "monitored continuously," and the animals were weighed weekly. After 28 days, the mice were killed by decapitation, gross pathological examinations were performed, and the liver, spleen, thymus, and kidney were weighed. Blood was taken at sacrifice for analysis; no baseline analysis was conducted prior to the beginning of exposure and no sampling was done at intermediate time points. Splenocytes were prepared for analysis of mitogen-stimulated lymphocyte proliferation, mixed lymphocyte response, and cell-

mediated cytolysis response.

Data were reported for 5 animals per group for each assay. During the 28-day exposure, no mortality or any overt clinical signs occurred in exposed mice. Phenol treatment had no effects on food or water consumption, or on body weight gain. Exposed mice had no gross lesions in the liver, kidney, spleen, thymus, lung, heart, and brain, and had no effect on organ weights for the liver, kidney, spleen, and thymus. There was a dose-related decrease in erythrocyte counts that was statistically significant at all doses (Table 5). Because hematocrit was decreased only at the high dose, the significance of the decrease in erythrocytes at the lower doses is unclear, particularly . The erythrocyte counts in all dosed groups were markedly lower than the historical control values provided by the animal distributor (Charles River Laboratories, 1986), although the hematocrit concentration in all groups was above the historical control mean. There was no effect on total or differential leukocyte counts. Interestingly, total white blood cells for all groups, including the controls, were below the historical control data provided by the distributor.

The study authors also reported effects on immune endpoints, but possible technical problems with the assays raise questions regarding these measurements. Although decreases in the absolute splenocyte lymphoproliferative responses to mitogens were observed at the high dose, this decrease is questionable, because the background level of proliferation in the high-dose group was much lower than in the other groups, and so the stimulation index (ratio of mitogen-induced to background proliferation) appears to be unaffected. This suggests that the splenocyte preparations (at least at the high dose) may have contained a significant percentage of nucleated red blood cell precursors (perhaps reflecting extramedullary hematopoiesis). If the total cell counts used to normalize the results contained these red blood cell precursors, rather than only nucleated proliferating cells, the observed apparent decreases in response would occur. The mixed lymphocyte response (proliferative ability of splenic lymphocytes in response to alloantigens) was also decreased at the high dose. The reliability of this finding is questionable, since there was no effect on the cytolytic response against tumor cells, and the cytolytic response is an important component of the mixed lymphocyte response. In separate experiments, the phenol-treated mice were injected with sheep red blood cells prior to sacrifice. At the two highest doses, the antibody production was decreased (as measured by serum titer of anti-sheep red blood cell antibody and by the number of IgM antibody plaque-forming cells).

Hsieh et al. (1992) also observed dose-related decreases in the concentration of several neurotransmitters and their metabolites in the brain, including levels of norepinephrine, indoleamine serotonin, dopamine, and their metabolites. The toxicological significance of these neurobiochemical findings is unclear, in the absence of a clear correlation to clinical effects.

Thus, this study found dose-related statistically significant decreases in red blood cells at all doses, but decreased hematocrit only at the high dose. Decreases in various immune parameters were reported at the high dose, but inconsistencies in the results raise questions about whether the observed effect was meaningful. Based on the decreased hematocrit and indications of suppressed immune function at the high dose, the mid dose (6.2 mg/kg-day) can be considered the study NOAEL, and the high dose (33.6 mg/kg-day) can be considered an equivocal LOAEL.

There is, however, considerable uncertainty regarding the reliability of these values.

In contrast to the minimal effects observed in these drinking water studies, gavage dosing with phenol produces severe toxicity, including liver and kidney pathology, and death at doses that cause only minimal effects when delivered in drinking water.

Dow Chemical Co. (1945) administered 0, 50, or 100 mg/kg phenol by gavage 5 days/week to 10 rats/group (sex and strain not reported) for 6 months (0, 35.7 or 71.4 mg/kg-day after adjusting for intermittent dosing). The dosing volume was not reported. Mortality occurred in 1/10, 4/10, and 4/10 rats in the control, low-, and high-dose groups. The study authors raised questions about whether the mortality was treatment-related, but it was not clear if they questioned whether the deaths were due to phenol, or whether they were due to gavage accidents. Other observed effects were slight cloudy swelling of the liver and of the tubular epithelium at the high dose, and slight tubular degeneration at the low dose. This unpublished study[4] is limited by the incomplete reporting of methods and results, but the low dose of 35.7 mg/kg-day appears to be a LOAEL.

In a series of toxicological screening tests, the systemic, neurological and developmental effects of phenol in F344 rats following acute and short-term oral exposure were examined (Narotsky and Kavlock, 1995; Berman et al., 1995; and Moser et al., 1995; MacPhail et al., 1995). In these tests, systemic and neurological effects were examined on the same animals following exposure by gavage to a single dose of phenol or to 14 consecutive daily doses. Developmental toxicity was also examined in pregnant rats that received phenol by gavage on gestation days 6-19. The dosing volume was 1 mL/kg (Moser et al., 1995; Narotsky and Kavlock, 1995).

In the acute toxicity study of this series, groups of eight female rats were given a single gavage dose (1 mL/kg volume) of phenol at 0, 12, 40, 120, or 224 mg/kg in water (Berman et al., 1995; Moser et al., 1995). A functional observational battery (FOB) evaluating autonomic and neuromuscular functions, activity, excitability, sensorimotor and physiological measures was conducted prior to the exposure and at approximately 4 and 24 hours after exposure. Immediately after the 24-hour FOB, the animals were sacrificed, blood samples were collected for serum-chemistry analyses, and the liver, kidneys, spleen, thymus and adrenals were weighed and subjected to histopathological examinations. Two rats (25%) died within 4 hours of exposure to 224 mg/kg, and one rat died 24 hours after exposure to 120 mg/kg phenol.
The only treatment-related effects observed were confined to these two dose groups, and included tremor, decreased motor activity, and kidney pathology (necrosis, protein casts, and papillary hemorrhage). Hepatocyte necrosis was also observed at 40 and 120 mg/kg, but not at 224 mg/kg. No other effects were reported at the lower doses, although the primary data were not provided.

[4]Although this unpublished study is not of high quality, and was not peer-reviewed, the study is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

In the short-term study, groups of eight female rats were given daily gavage doses of phenol in water at 0, 4, 12, 40 or 120 mg/kg-day for 14 consecutive days (Berman et al., 1995; Moser et al., 1995). As in the acute study, the FOB was conducted prior to exposure, as well as on days 4 and 9 (before the daily dose), and approximately 24 hours after the last dose. After the last FOB, blood samples were collected for serum chemistry analyses, and internal organs were removed, weighed, and subjected to histopathological examinations. All rats administered the high dose died during the study, but deaths occurred over the entire dosing period. Tremor was also seen in the high-dose (120 mg/kg) group immediately after the first administration, but not after subsequent treatment. Vacuolar degeneration of the liver, kidney necrosis and protein casts, and "necrosis or atrophy of spleen or thymus" were reported at 40 mg/kg-day. The increased incidences were not large enough to be statistically significant; the statistical power of the study was also low, with only 8 rats/group. Additional information on this study is available from a preliminary abstract (Schlicht et al., 1992) and from a recent WHO (1994) review. According to these sources, the renal pathology consisted of 3/8 rats with renal vascular stasis, 2/8 rats with tubular degeneration in the papillar region, and 1/8 with protein casts in the tubules. Based on personal communication with an author, WHO (1994) stated that the pathology report attributed the renal findings to decreased vascular perfusion. There were also slight, but not statistically significant, decreases in motor activity at 40 mg/kg-day. The only statistically significant effect in this group was increased rearing in the post-exposure measurement. The only effect at 12 mg/kg-day was "necrosis or atrophy in the spleen or thymus" in 1/8 rats. Based on the liver, kidney, and thymus/spleen pathology, which is rarely observed in control animals in 2-week studies, and based on the decreased motor activity, the second dose (40 mg/kg-day) was the study LOAEL, and the mid dose of 12 mg/kg-day was the study NOAEL.

4.2.2 Inhalation

The laboratory animal inhalation data for phenol are very limited, with only one 2-week toxicity study (Huntingdon, 1998) conducted using modern methodology and documentation. Although a subchronic study conducted with multiple laboratory animal species is available (Sandage, 1961), this unpublished study tested only one concentration and was insufficiently documented for definitive risk assessment purposes. Other short-term (Dalin and Kristoffersson, 1974) or subchronic (Deichmann et al., 1944) inhalation toxicity studies are limited by the short duration, inadequate documentation, or lack of a modern exposure protocol. Nonetheless, the data are consistent that the respiratory tract, kidney, lungs, and nervous system are targets of inhalation exposures.

In conducting dosimetric conversions from animal studies to human exposure scenarios, U.S. EPA (1994b) classifies gases according to their water solubility and reactivity. Category 1 gases are highly water soluble and/or rapidly reactive, and do not penetrate the blood. Category 3 gases are water insoluble and uptake from the lungs is limited by perfusion. Category 2 gases are intermediate between these two groups. They are moderately water soluble and rapidly reversibly reactive or moderately to slowly irreversibly metabolized in the respiratory tissue. Based on the chemical/physical properties of phenol (moderately water soluble [Table 1] and moderately reactive [based on the evidence of irritation and corrosivity seen following direct contact]),

phenol can be considered to be classified as a Category 2 gas. This conclusion is supported by the finding of both respiratory effects (from direct contact) and systemic (extrarespiratory) effects (from absorbed phenol) following inhalation exposure, as described below. Because the equations for the regional gas dosimetry ratio (RGDR) for Category 2 gases are currently undergoing EPA reevaluation (equations 4-29 through 4-44, pages 4-52 through 4-57 of U.S. EPA, 1994b), dosimetric adjustments for extrarespiratory effects were made using the Category 3 equations (Equation 4-48, page 4-60 of U.S. EPA, 1994b), in which the RGDR is based on the blood:air partition coefficient for the chemical in the experimental animal species and in humans. No data on the blood:air partition coefficient for phenol in laboratory animals or humans were located. Therefore, the default value of 1 for the ratio of the laboratory animal to human partition coefficient was used, and the human equivalent concentration (HEC) for systemic effects was the same as the duration-adjusted concentration. HECs for respiratory tract effects were calculated using the equations of U.S. EPA (1994b) for a Category 1 gas (Equations 4-17 through 4-28, pages 4-47 through 4-51). When the EPA reanalysis is complete, revised dosimetric conversions may be calculated. The inhalation toxicity data for phenol are summarized in Table 6.

The acute toxicity studies support the findings of the short-term and subchronic studies that the respiratory tract and nervous system are targets of inhaled phenol. For example, tremors were seen in rats and guinea pigs exposed to 187 or 540 ppm (720 or 2080 mg/m^3) phenol for 30 minutes in a whole-body inhalation chamber (UBTL, 1991). By contrast, no tremors were observed in rats exposed via nose-only inhalation at 25 ppm (96 mg/m^3) for 6 hours (Dow Chemical Co., 1994). Phenol also caused sensory irritation in mice, as evidenced by decreased respiratory rate (De Ceaurriz et al., 1981). The concentration associated with a 50% decrease in rate (RD_{50}) was estimated to be 166 ppm (639 mg/m^3). No acute lethality studies were identified for phenol following exposure by the inhalation route.

In an unpublished 2-week inhalation study conducted according to GLP guidelines (Huntingdon, 1998), groups of 20 F344 rats per sex were exposed nose-only to actual concentrations of 0, 0.52, 4.9, or 25 ppm phenol (0, 2.0, 18.9, and 96.2 mg/m^3) 6 hours/day, 5 days/week for 2 weeks.[5] The duration-adjusted concentrations were 0, 0.36, 3.4, and 17 mg/m^3, respectively. The animals were observed twice daily for mortality and abnormal clinical signs. Animal body weights and food consumption were recorded twice pretest, weekly thereafter, and just prior to sacrifice. At the end of 2 weeks of exposure, 10 rats of each sex in each group were sacrificed. The rest of the rats were sacrificed after 2 weeks of recovery. Blood samples were collected just prior to sacrifice for hematological (including differential leukocyte count) and biochemical examinations. Gross pathological evaluations were conducted on all of the animals, and organ weights were determined. Histopathological examinations were conducted on the liver, kidney, and respiratory tract tissues (including 3 sections of the lungs with mainstem bronchi, the

[5]This study has not been peer-reviewed, but it was well-conducted, and is well-documented, contributing useful information to the hazard identification and dose-response portions of the assessment. The study duration falls between the existing EPA guidelines, although the overall study design and endpoints evaluated were similar to the guidelines for a subchronic inhalation study. The study was designed to comply with the U.S. EPA Enforceable Consent Agreement for Phenol.

pharynx, and 3 sections of the nasal turbinates) of the control and high-exposure groups; the spleen of mid-concentration females was also analyzed.

During the exposure, one male rat in the low-concentration group accidentally died from a trauma caused by turning itself within the nose-only restraint tube. All of the other rats survived until sacrifice at the end of the 2-week exposure or 2-week recovery periods. During exposure and recovery, there were no treatment-related changes in weekly physical examinations, body weight, weight gain, or food consumption. The study authors reported no effect on clinical signs. However, there was a concentration- and duration-related increase in the incidence of a red nasal discharge in the males. The incidence was 0/20, 0/20, 3/20, and 4/20 at 0, 0.52, 4.9, and 25 ppm, respectively, in the first week, and 0/20, 0/20, 7/20, and 10/20 in the second week of exposure. In an analysis done for this assessment, the incidence at the mid and high concentrations was statistically significant, using the Fisher Exact test. In females, nasal discharge was seen in 1/20 at the low concentration and 3/20 at the mid concentration in the second week, but no discharge was reported in high-concentration females. Prior to exposure, the discharge was observed in only a single control male and a single high-concentration female. Based on the exposure chamber design, it does not appear that the discharge was an artifact of the rats' noses being in contact with phenol condensate on the chamber walls. Instead, this assessment considered the discharge to be a treatment-related sign of irritation.

Hematological and biochemical examinations showed slight but statistically significant increases in prothrombin time at the low concentration only, and in albumin concentration in high-exposure females; these changes were not considered of biological significance. No other significant changes in hematology or biochemistry were observed. The only statistically significant changes in organ weights were the decreases in liver-to-body, spleen-to-body and spleen-to-brain weight ratios in high-concentration females. Gross pathological and microscopic examinations of these organs did not exhibit any differences from the controls. Although there were a number of histopathology findings in the respiratory tract (e.g., inflammatory cells in the nasolacrimal ducts, alveolar macrophages, and eosinophilic and basophilic material), these findings occurred at similar incidences in the control and exposed groups. The lesions reported were also those typically seen in control animals. The only lesion of concern was minimal to slight lung hemorrhage, which was reported in 4/10 control males and 6/10 high-exposure males at the terminal sacrifice. However, there was no clear concentration-related increase in incidence or severity, this lesion was not found in the females, and this lesion was not seen in exposed animals post-recovery or in control animals. Thus, it appears that the only effect of concern in this study is the red nasal discharge, which was observed in males with a concentration- and duration-related incidence. Although no supporting histopathology was observed in a thorough examination, the nature of the discharge and the increased incidence with duration suggests that it may be an effect that would progress to visible lesions at longer exposure durations. Based on the red nasal discharge in males, the low concentration (2 mg/m^3) was a NOAEL and the middle concentration (18.9 mg/m^3) was a LOAEL. The NOAEL (HEC) for this extrathoracic effect was 0.05 mg/m^3, and the LOAEL (HEC) was 0.5 mg/m^3.

Deichmann et al. (1944) conducted subchronic inhalation studies of phenol toxicity in rabbits, rats, and guinea pigs. Twelve guinea pigs, 6 rabbits, and 12 rats (strain and sex not reported) were exposed (whole-body) in a single exposure chamber to phenol vapor at "a concentration ranging from 0.1 to 0.2 mg /L (100-200 mg/m^3)" for 7 hours/day, 5 days/week for 6 weeks, 13 weeks, or approximately 11 weeks, respectively. The actual exposure concentration apparently could not be controlled more precisely. Using the midpoint of 150 mg/m^3 as the exposure concentration, the duration-adjusted concentration was 31 mg/m^3. Among the three tested species, the guinea pig was the most sensitive, and the rat was the least sensitive to phenol exposure.

Deaths were observed in 5/12 guinea pigs during the 6-week exposure period. Other signs of toxicity in the guinea pigs included decreased activity during the first week, and respiratory difficulties and paralysis of hind quarters after 4 weeks of exposure. Histopathological evaluations revealed lesions of the lungs (pneumonia and bronchitis), heart (inflammation, fibrosis, and necrosis), liver (fatty changes and necrosis), and kidneys (tubular degeneration and edema). At the end of exposure, the surviving guinea pigs had a concentration of 1.0 mg free phenol/100 mL blood, and 0.4 mg conjugated phenol/100 mL blood. (Details on the analytical procedures used to measure phenol in blood were not reported.). No deaths or clinical signs of toxicity were observed in the rabbits exposed for 13 weeks, but lobular pneumonia and fibrosis was observed in these animals. Histopathology lesions in the heart, kidney, and liver were similar to, but less severe than, those reported in the guinea pigs. After 37 days on study, the rabbits had a concentration of 0.5 mg free phenol/100 mL blood, and 0.7 mg conjugated phenol/100 mL blood; similar concentrations were observed at the end of the exposure period.

The rats did not show any clinical signs of toxicity during the 74-day exposure period (approximately 11 weeks), and there was no histopathological evidence of any effect. Blood phenol levels were not reported for the rats, but an analysis of carcass homogenate found 0.2 mg free phenol and 0.35 mg conjugated phenol per 100 g carcass homogenate. These levels were reported to be within the normal range in unexposed rats. This study is limited by the use of only one exposure concentration, the absence of controls, the inadequate control of exposure levels, and the absence of reporting of the primary data. However, the results do appear to show that rats are much less sensitive to the inhalation effects of phenol than rabbits or guinea pigs. Based on a comparison of blood levels, however, it appears that the interspecies differences are due to both toxicokinetic and toxicodynamic differences.

In an unpublished 90-day study, groups of 10 male Rhesus monkeys, 50 male Sprague-Dawley rats, and 100 male albino mice were exposed to average phenol concentrations of 0 or 4.72 ppm (18.2 mg/m^3) continuously for 90 days (Sandage, 1961).[6] Exposure was interrupted for

[6]Although this unpublished study is not of high quality, did not meet EPA guidelines, and was not peer-reviewed, the study is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

14 hours on day 39, and for 36 hours on days 68-69. The frequency of monitoring of the test atmosphere was not reported, but the phenol concentration was reported to remain in the desired range of 4.5-5.5 ppm "after the first three days." No further information on the concentrations during the first three days was reported.

During the exposure, no deaths were observed in the test animals. Body weight gain in mice was comparable to that in controls, but was slightly higher in exposed rats and monkeys. A complete hematological examination showed no significant changes in the three test species following phenol exposure. Blood biochemistry (alkaline phosphatase, cholinesterase, amylase, lipase, and glutamic oxalacetic transaminase) was evaluated in monkeys only. Urinalysis was apparently conducted in all species, but kidney function tests (urine volume and specific gravity) was evaluated only in monkeys and rats. The study authors reported that there were no effects on any of these endpoints, but did not provide any supporting data. At the end of the exposure period, "approximately half" of the animals underwent a stress test, in which the animals swam in a smooth-walled tank until exhausted. These animals were sacrificed immediately after the test, and the other animals were held for a 2-week recovery period prior to sacrifice. Histopathological evaluations were conducted on only 5-8 organs (including the liver, kidney, and lung). It appears that all of the monkeys and about half of the rats and mice were evaluated, although it is not clear whether some of the rodents were evaluated after the recovery period.

The study authors considered the histopathology findings "essentially negative," and did not provide any description of the observed lesions or the number of animals examined histopathologically. Liver and kidney pathology was observed in 30% and 20%, respectively, of the monkeys, compared to 0% of the controls. However, the authors did not consider these changes to be significant and noted that 6/7 reports of pathology in monkeys were considered "minimal or doubtful." Liver and kidney pathology was also reported in 20% of phenol-exposed rats, compared to 0% of the controls, and lung pathology was reported in 20% of the phenol-exposed mice, compared to 6% of the controls. The incidences of liver and kidney pathology in the rat, and lung pathology in the mouse, was statistically significant, based on a Fisher's exact test done for this assessment. Although the incidence of lung pathology was not affected in monkeys and rats, a relatively high incidence of lung pathology in the control animals (30% and 65%, respectively) decreased the sensitivity of the evaluation. No other significant pathological changes were reported in the test animals. While the authors concluded that there was no evidence that phenol exposure resulted in significant damage, there is some indication of liver, kidney, and lung pathology in this study, but the inadequate reporting precludes the determination of whether there was a treatment-related effect. For the purposes of this assessment, the single exposure level tested, 18.2 mg/m^3, should be considered a LOAEL, although it might be considered a minimal LOAEL if additional histopathology data were available. The LOAEL(HEC) for the kidney and liver lesions is also 18.2 mg/m^3. In the absence of additional information on the nature of the lung lesions, the LOAEL (HEC) for the lung can not be determined. The study is also limited by the poor control of exposure levels and limited reporting of effects.

In a study published by Dalin and Kristoffersson (1974), 7 white rats of an in-house strain were exposed to phenol at a concentration of 100 mg/m^3 continuously for 15 days. There is some

uncertainty in this exposure measure, however, since the exposure chamber was not set up according to modern designs, and it does not appear that continuous monitoring of exposure levels was conducted. Unexposed rats (n=11-12) were used as the controls. Nervous system effects were observed from the first day after the start of exposure. These effects progressed from increased activity, to imbalance, twitches, and disordered walking rhythm on days 3-4. These signs disappeared by day 5 and were replaced with sluggish behavior until the end of the exposure. A tilting-plane test was conducted before and after exposure in both groups, and a significant effect was observed on the exposed rats. There were no significant changes in food intake or water consumption during the exposure period. Although there was no significant difference in body weight of the exposed group compared to the controls, the average body weight of the exposed group decreased during exposure, while the controls gained weight. The serum biochemical evaluations showed large, statistically significant increases in SGOT, SGPT, LDH, and glutamate dehydrogenase activities, indicating liver damage. Plasma potassium and magnesium levels were also increased. Although the significance of these changes is unknown, the study authors suggested that the increased magnesium levels may have caused some of the nervous system effects. Hemoglobin and hematocrit were unaffected. No histopathology examination was conducted. Based on the observed nervous system effects, as well as on the serum enzyme changes indicating liver damage, the only exposure concentration was a LOAEL. The LOAEL(HEC) is 100 mg/m^3, but the actual exposure measurement is of low quality.

4.2.3 Dermal

Phenol is quite irritating, and dermal exposure to liquid phenol can result in inflammation and necrosis of the skin (Conning and Hayes, 1970; Patrick et al., 1985; Pullin et al., 1978). As discussed in Section 3.1, phenol is readily absorbed from dermal contact with phenol liquid or phenol vapor, so systemic effects can also result from dermal exposure. Several acute lethality assays have been reported. Conning and Hayes (1970) reported a dermal LD_{50} of 669.4 mg/kg for undiluted phenol applied for 24 hours to the skin of female Alderly Park rats. Acute dermal toxicity appears to be dependent on the concentration of phenol, with increased lethality observed with decreased concentration when the same total dose is applied (Deichmann and Witherup, 1944; Conning and Hayes,1970). In rabbits, dermal LD_{50} values of 850 mg/kg in rats (Flickinger, 1976) and 1400 mg/kg (phenol concentration not specified) were reported. In addition to lethality, renal effects (severe hemoglobinuria and hematin casts in the tubules), cardiovascular effects (cardiac arrhythmias and ventricular tachycardia), and neurological effects (severe muscle tremors, marked twitching, generalized convulsions, loss of consciousness, and prostration) were observed at 107.1 mg/kg in female Alderly Park rats following dermal exposure to undiluted phenol for 24 hours (Conning and Hayes, 1970). A similar array of effects has been reported in humans following accidental dermal exposures to large volumes of phenol (ATSDR 1998).

4.3 Reproductive/Developmental Studies

No studies of the reproductive or developmental toxicity of phenol following inhalation exposure of laboratory animals were located. Several developmental toxicity studies in rats and mice conducted via the gavage route are available (Argus Research Laboratories, 1997; NTP,

1983a; NTP, 1983b; Narotsky and Kavlock, 1995); the only developmental effect reported in these studies was decreased fetal body weight. In a two-generation drinking water study in rats (IIT Research Institute, 1999), decreased pup weight and decreased survival of pups preculling were observed, but these effects appear to be secondary to decreased water consumption.

In an unpublished two-generation reproductive toxicity study following modern GLP guidelines (IIT Research Institute, 1999), 30 Sprague-Dawley rats/sex/group were exposed to 0, 200, 1000 or 5000 ppm phenol in drinking water.[7] Parental (P1) rats were given phenol for 10 weeks prior to mating, during a 2-week mating period, throughout gestation, lactation, and until sacrifice. The males were sacrificed after successful mating. All of the P1 females were allowed natural parturition, and were sacrificed at F1 weaning. The study authors calculated that the average daily phenol intake during week 10 was 0, 14.7, 70.9, and 301.0 mg/kg-day for P1 males, and 0, 20.0, 93.0, and 320.5 mg/kg-day for P1 females. For the F1 generation, the average phenol intake during week 10 was 0, 13.5, 69.8, and 319.1 mg/kg-day for males and 0, 20.9, 93.8, and 379.5 mg/kg-day for females. The F1 generation (20 rats/sex/group) was treated following a protocol similar to that used for the P1 generation, and F2 pups were sacrificed after weaning, on postnatal day (pnd) 22. During treatment, rats were monitored for mortality, clinical signs, body weight, and food and water consumption. At sacrifice, the animals were necropsied and reproductive organs from 20 animals/sex in the control and high-dose groups from the P1 and F1 generations were examined microscopically. In addition, the spleen, thymus, liver, and kidneys from 10 randomly selected P1 and F1 animals of each sex in the control and high-dose groups were also examined.

Most of the treatment-related changes in P1 rats were observed in the high-dose groups. No treatment-related mortality was observed in P1 rats of either sex. Three high-dose F1 female pups died shortly after weaning. The deaths appeared to be associated with decreased water consumption associated with poor palatability, since these pups refused to drink the water containing the phenol. No other treatment-related mortality was reported in the F1 generation. The only significant observed clinical sign was redness around the nose fur in high-dose males and females of both generations. This redness may have been due to the water containing the phenol spilling onto the fur around the nose and mouth, and causing irritation. A significant decrease in water consumption was observed throughout the study in P1 animals of both sexes (up to 23% for males and up to 39% for females), and was attributed to poor palatability. Decreased water consumption in the F1 rats at the high dose was of a similar or larger magnitude. The low water consumption at the high dose was accompanied by decreased body weights compared to the controls (9% in P1 males and 16% in P1 females at sacrifice).

High-dose F1 and F2 pups had decreased body weights compared to the controls at birth and the difference from the controls were larger by pnd 21. The study authors noted that pups

[7]This study has not been peer-reviewed, but it was conducted according to EPA guidelines for reproduction and fertility effects, is well-documented, and contributes useful information to the hazard identification and dose-response portions of the assessment.

began drinking the water prior to weaning, and that the decreased water consumption compared to controls was also observed in the high-dose pups. Decreased absolute organ weights and increased relative organ weights were observed for a number of organs at the high dose in both the P1 and F1 generations. These changes likely reflected the lower body weight and overall dehydration in these groups. F1 females had a statistically significant, dose-related decrease in uterine weights at all doses. The study authors suggested that this decrease may have been related to a lower incidence of uterine dilatation at the high dose, but they did not consider the small decreases in the number of rats in estrus (16/24, 15/24, 13/25, 9/22 in the control, low-, mid- and high-dose groups) sufficient to account for the decreased uterine weight. Dose-related statistically significant decreases in absolute adrenal and spleen weights were also observed in the mid- and high-dose F1 males, but there was no effect on these organ weights at any dose in the P1 males, and P1 females were only affected at the high dose (where decreased body weight would have played a role). The pathological examinations showed no treatment-related lesions in the kidneys, spleen, liver, thymus, or reproductive organs. An immunotoxicity screen conducted with 10 male P1 rats/group found no significant effects on spleen weight, cellularity, or antibody-forming cells for any test group compared to the control group; the expected results were found with a positive control group. Complete hematological evaluations (including hematocrit, erythrocyte count, and differential white cell count) and serum biochemical evaluations were conducted on 8-10 P1 males/group prior to sacrifice. The only significant change in these evaluations was increased blood urea nitrogen (BUN) in the high-dose group. Since this change was not accompanied by increased creatinine, and there was no associated kidney pathology, the BUN increase was not considered to be biologically significant. The study authors also noted that all but one of the high-dose BUN values were within the control range.

There was no effect on fecundity or fertility in either generation. In addition, there was no effect on other indicators of reproductive toxicity, including the frequency of estrus, testicular sperm count, sperm motility and sperm morphology. The survival of the high-dose F1 pups was significantly decreased on pnd 4 (pre-culling), although there was no effect on overall F1 pup survival. In the F2 generation, high-dose pup survival was significantly decreased throughout the lactation period. This decreased survival of both generations of pups was likely secondary to the decreased maternal water intake, and associated decreases in milk production. In the F1 generation, delayed vaginal patency and delayed preputial separation were observed at the high dose. Since these developmental landmarks are correlated with body weight, the delays were likely related to the reduced body weight compared to the controls. However, the study authors did not test this suggestion by reporting the body weight at these developmental landmarks.

Thus, all of the systemic and reproductive effects of phenol in this study occurred at the high dose, and appear to be secondary to decreased water consumption due to poor palatability, rather than a toxic effect of phenol. Based on the decreased parental and pup body weight (compared to the controls), and decreased pup survival, the high dose is a LOAEL. The study NOAEL is 70.9 mg/kg-day (based on the NOAEL corresponding to the lowest LOAEL in this study, in P1 males).

In a well-conducted unpublished developmental toxicity study conducted according to GLP guidelines (Argus Research Laboratories, 1997), pregnant Crl:CDRBR VAF/Plus Sprague-Dawley rats (25/group) received phenol by oral gavage on gestation days (GD) 6 through 15.[8] Dosing was three times daily with 0, 20, 40, or 120 mg phenol/kg/dosage, using a dosing volume of 10 mL/kg. The corresponding daily doses were 0, 60, 120, and 360 mg/kg-day. The study authors noted that the test material was 90% phenol USP; the authors adjusted the dosage calculations for test material purity. The exposed dams were observed twice a day for viability, and daily for clinical signs, abortions, and premature deliveries. In addition, the maternal body weights were recorded every day, and food consumption was also recorded periodically (every 1-2 days). The rats were sacrificed on GD 20, and gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. Each fetus was weighed, sexed, and examined for gross external alterations. One half of the fetuses were examined for soft tissue alterations, and the rest were examined for skeletal alterations.

One high-dose dam died on gestation day 11. The study authors attributed this death to the phenol treatment, because it occurred only at the high dose, although there were no adverse clinical observations and no abnormal necropsy findings in this animal. Other high-dose animals exhibited excess salivation and tachypnea (rapid breathing). There were no other treatment-related clinical observations, and no treatment-related necropsy findings. Dose-dependent decreases in body weight of the exposed animals compared to the controls were observed. Statistically significant decreases compared to controls in both maternal body weight (8%) and body weight gain (38% for GD 6-16) were observed at the high dose; although a statistically significant decrease in body weight gain (11%) was observed at the mid dose, the decrease (relative to controls) in absolute maternal weight at the end of dosing (3%) was not statistically significant. Dose-dependent decreases in food consumption were also observed during the dosing period. Fetal body weights in the high-dose group were significantly lower than the controls, by 5-7%. Delayed skeletal ossification was observed at the mid and high doses, and was likely related to decreased fetal body weights; the only statistically significant change was a decrease at the high dose in the average number of metatarsal ossification sites per fetus per litter. There were no other treatment-related effects on uterine contents, malformations, or variations. The maternal NOAEL was 60 mg/kg-day, based on small decreases in maternal body weight gain at 120 mg/kg-day, and the developmental NOAEL was 120 mg/kg-day, based on decreased fetal body weight and delayed ossification at 360 mg/kg-day.

In a well-designed developmental toxicity study (NTP, 1983a), timed-mated CD rats were administered phenol by gavage at 0, 30, 60 or 120 mg/kg-day in 5 mL/kg distilled water on GD 6

[8]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for developmental toxicity studies, is well-documented, and contributes useful information to the hazard identification and dose-response portions of the assessment. The study was designed to meet the U.S. EPA Pesticide Assessment Guidelines, Subdivision F, 83-3.

to 15, and sacrificed on GD 20. Females were weighed on GD 0, 6 through 15 (prior to daily dosing) and 20 (immediately following sacrifice), and were also observed during treatment for clinical signs of toxicity. A total of 20-22 females/group were confirmed to be pregnant at sacrifice on GD 20. The dams were evaluated at sacrifice for body weight, liver weight, gravid uterine weight, and status of uterine implantation sites. Live fetuses were weighed, sexed, and examined for gross morphological abnormalities and malformations in the viscera and skeleton. Results of this study did not show any dose-related signs of maternal toxicity or any clinical symptoms of toxicity related to phenol treatment. The number of implantation sites was slightly higher in the dosed groups, but this change could not be treatment-related, since implantations in this strain take place prior to GD 6 (prior to dosing). There was also no effect on live fetuses, sex ratio, malformations, or variations. There was, however, a clear dose-related downward trend in fetal body weight, although the changes at the two lower doses are small and the effect was statistically significant only at the high dose (Table 7). Fetal body weights in the high-dose group were 93% of the average in the control group; fetal body weights were not reported separately for males and females. Historical control data from the supplier reports the average fetal body weight in this strain as being well below the weight in the high-dose group (Charles River, 1988). (Concurrent control weight was 4.14 g, high-dose weight was 3.84 g, and historical control weight was 3.39 g.) The litter size in the high-dose group was also somewhat higher (not statistically significant) than in the controls, possibly contributing to the smaller fetal weight at the high dose. As shown in Table 7, the total pup burden (total fetal weight) and the gravid uterine weight were highest in the low-dose group, followed by the values in the high-dose group, and these values were both higher than those in the control group. In addition, the treatment period maternal weight gain was very similar in the control and high-dose groups (but higher in the low-dose group), but the absolute maternal weight gain (i.e., adjusted for the gravid uterine weight) was much lower in the high-dose group than in the control. The results from the low-dose group suggest that the dams could have borne a somewhat higher burden of the total *in utero* package. However, the results also indicate that the dams were near the limit of what they could carry, based on the lower absolute weight gain, but unaffected treatment-period weight gain, in the high-dose group. There were no dose-related signs of maternal toxicity, or any clinical symptoms of toxicity related to phenol treatment in this study. Based on these considerations and the potential for the decreased fetal weight to reflect primarily the larger litter size, the decreased fetal weight in this study could be considered an equivocal LOAEL. Thus, based on decreased fetal body weight, the mid dose in this study of 60 mg/kg-day was a NOAEL for developmental toxicity and the high dose of 120 mg/kg-day was an equivocal LOAEL. The high dose (120 mg/kg-day) was a maternal NOAEL.

The preliminary rat developmental toxicity studies (NTP 1983a) found that phenol toxicity is increased by the use of small dosing volumes. For example, when phenol was administered by gavage on GD 6-15 to pregnant CD rats at doses of 0, 125, 160, 200, or 250 mg/kg-day in a volume of 1 mL/kg, the mortality was 0% (0/9), 70% (7/10), 78% (7/9), 100% (9/9) and 100% (9/9), respectively. The deaths were preceded by dose-related signs of toxicity, including tremors, convulsion, and respiratory distress; mottled liver and congested lungs were found on necropsy. In contrast, when the same doses were administered in a volume of 5-7.5 mL/kg, the respective mortality was only 0% (0/24), 0% (0/5), 17% (1/6), 17% (1/6), and 71% (5/7), respectively. Based on these results, a volume of 5 mL/kg was used in the main developmental

toxicity study. In preliminary toxicity studies conducted with doses of 60-250 mg/kg-day in a volume of 5-7.5 mL/kg, decreased maternal body weight gain (or body weight loss) during dosing was observed at 160 mg/kg-day and up, doses at which mortality was also observed. In addition, tremors were observed sporadically in the phenol-dosed groups, without any clear dose-response. There were no treatment-related changes in prenatal viability, fetal sex ratio, or fetal structural development. The study authors stated that, when results of all of the preliminary studies were pooled, a statistically significant trend of decreasing fetal weight was observed, but there were no statistically significant differences from controls in pairwise analyses. The power of the pairwise tests was limited because only 4-6 litters were produced in the dose range 100-200 mg/kg-day.

In a standard mouse developmental toxicity study (NTP, 1983b), phenol was administered by gavage in water at 0, 70, 140, or 280 mg/kg-day on GD 6 to 15, in a volume of 10 mL/kg. Groups of 31 to 36 plug-positive female CD-1 mice were used in each treatment group. The pregnancy rate in the controls was only 83%; the pregnancy rate in dosed animals ranged from approximately 83% in the low- and mid-dose groups to 71% at the high dose. In addition, 4/36 high-dose mice died; no deaths occurred in any other groups. The average maternal body weight gain during treatment was statistically significantly reduced at the high dose, as was the maternal body weight at terminal sacrifice on GD 17 (by 10%, compared to the control group). In addition, tremors were observed at the high dose throughout the dosing period. As in the rat study, there was a highly statistically significant decrease in fetal body weight per litter (18%) at the high dose. An increased incidence of cleft palate was also reported at the highest dose level, although the incidence was not significantly different from the other groups and there was no statistically significant increase in the incidence of litters with malformations. There was no other evidence of altered prenatal viability or structural development. Thus, the high dose of 280 mg/kg-day was a maternal FEL, based on the observed deaths; tremors and decreased body weight also occurred at this dose. The high dose was also a developmental LOAEL, based on decreased fetal body weight (accompanied by a possible increase in the incidence of cleft palate) in the fetuses, effects which were likely secondary to the severe toxicity in the dams. The study NOAEL for maternal and developmental toxicity was 140 mg/kg-day.

The series of oral screening studies mentioned above (Narotsky and Kavlock, 1995; Berman et al., 1995; and Moser et al., 1995; MacPhail et al., 1995) also included a developmental toxicity screening study. In this study, groups of pregnant F344 rats (15-20 animals/group) were given phenol at doses of 0, 40, or 53.3 mg/kg by gavage once daily on gestation day 6-19, and then sacrificed on postnatal day (pnd) 6 (Narotsky and Kavlock, 1995). The dosing volume was 1 mL/kg. Pups in each litter were examined and counted on pnd 1, 3, and 6, and were weighed on pnd 1 and 6. Uterine implantation sites were counted after the dams were killed. Only minimal quantitative data were presented. No maternal deaths were observed. The authors reported that phenol treatment altered respiration (rales and dyspnea) at both dose levels, but no quantitative data were presented. Decreased (but not statistically significant) maternal body weight (compared to the controls) and decreased (statistically significant) maternal body weight gain were also reported at both doses, but there was no clear dose-response. No statistically significant evidence of developmental toxicity due to phenol exposure was observed. The only evidence of developmental toxicity came from dams exhibiting severe respiratory signs. These signs included

a dose-dependent increase in full-litter resorptions (one at the low dose and two at the high dose) and an excessive incidence of perinatal mortality and reduced pup weights on pnd 1 in one litter at the high dose. However, these changes as a group were not significantly different from the controls. Nonetheless, the respiratory effects from oral dosing reported in this study are of interest, particularly since they were not reported in the related study of systemic toxicity (Moser et al., 1995). This difference may reflect differences in the completeness of the study reporting. Alternatively, it may suggest that pregnant females may be more sensitive to the toxic effects of phenol than nonpregnant females.

4.4 Other Studies

4.4.1 Initiation/Promotion Studies, Other Short-term Tumorigenicity Assays, and Cancer Mechanism Studies

Several studies have tested the promotion potential of dermally-administered phenol. These studies have observed that phenol promotes tumors initiated with DMBA, but the phenol doses tested caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959), and thus were well above the MTD. One study (Wynder and Hoffman, 1961) observed promotion of DMBA-initiated tumors by dermally-administered phenol at a concentration that caused "no toxic reactions."

Salaman and Glendenning (1957) conducted an initiation/promotion study in which groups of 20 male "S" strain mice were initiated with a single dermal treatment with dimethyl-benzanthracene (DMBA), and promoted with dermal treatment with 0.5 mg/mouse phenol in acetone, using two different treatment *concentrations* for the *same applied dose*. The phenol was applied beginning 3 weeks after the DMBA application, either for 24 weeks in a volume of 0.025 mL as a 20% solution, or for 32 weeks in a volume of 0.1 mL as a 5% solution (rotating the weekly applications among four application sites for both concentrations). The study did not report whether the application site(s) were covered or whether the animals were restrained from licking the site. The high concentration produced local ulceration that healed just in time for the next treatment 4 weeks later, while the low concentration produced only transient light crusting that tended to decrease as the experiment progressed. It is unclear how severe the skin effects would have been if the low concentration were repeatedly applied to the same site, rather than rotating among four sites. Tumors were observed in both treatment groups, with a shorter time to first tumor and a higher tumor burden in the group treated with the higher phenol concentration. A few histologically confirmed malignant tumors (primarily squamous epitheliomas) were observed in both groups. In mice that underwent the same phenol treatment, but were not pretreated with DMBA, seven papillomas were observed at the high concentration. No tumors were observed at the low concentration, even though the weekly dose was the same, and the total dose per mouse was higher (since the duration was longer). There was no control group and no DMBA-only group, but the absence of tumors at the low concentration indicates that the observed tumors were phenol-related. The authors noted that the observed tumors might have been related to the significant skin injury produced by phenol. This suggestion is supported by the strong effect of the concentration of applied phenol at the same total dose.

Boutwell and Bosch (1959) conducted a series of initiation/promotion studies with different strains of mice. The mice were pretreated with a single application of 75 g DMBA in benzene followed by 5% or 10% phenol (1.25 or 2.5 mg per application) in benzene, or with either dose of phenol alone, twice weekly for 52 to 72 weeks. An additional group received DMBA alone, apparently followed by benzene vehicle, although there is some inconsistency between the text and the summary tables regarding whether the control group received the benzene vehicle. At the high phenol dose, dermal treatment with phenol resulted in decreased body weight (compared to the controls) and decreased survival. Skin wounds, hair loss, and reparative hyperplasia were also seen at the high dose, with the wounds predominantly seen in the first 6 weeks of treatment. By contrast, the authors stated that there was no evidence of ill effects of 5% phenol, except for its promoting activity. This statement was based on external observation; no histopathology was conducted. There was a dose-related increase in papillomas and in carcinomas in the groups initiated with DMBA and promoted with phenol. Increased papillomas were also observed in one strain treated with the high dose of phenol alone. There was evidence of decreased activity when phenol was further purified, indicating that the activity is not due to a contaminant. Since the benzene vehicle is a defatting agent, it is unclear whether it would have contributed to the effect of phenol.

Wynder and Hoffmann (1961) also found that dermally applied phenol is a promoter. Female Swiss mice (28-30/group) were initiated with a single application of DMBA, followed by treatment with 5% phenol three times weekly, or 10% phenol two or three times weekly. The dilution vehicle was not reported. "No toxic reactions" were reported at 5% phenol, although the higher concentration was reported to be "rather toxic." Treatment was for 12 months, and the mice were observed for an additional 3 months; the percentage of animals with papillomas and with cancers was recorded monthly. At 10 months, papillomas were seen in 33% of the low-dose group and >80% of the high-dose group; cancer was seen in 3% of the low-dose animals and 30-60% of the high-dose groups. By contrast, there were no papillomas or cancers in female Swiss mice treated with phenol alone, and only 10% papillomas (no cancer until week 12, and only 7% of the animals had cancer at study termination) in the mice treated with DMBA alone. Survival decreased markedly after week 10 in the high-dose groups, but not the other groups. In another experiment, the onset of tumor formation was much earlier in mice treated with 0.005% benzo[a]pyrene three times weekly plus 5% phenol twice weekly, than in mice treated with benzo[a]pyrene alone. Papillomas were observed by the second week in the groups receiving benzo[a]pyrene and phenol, and were present in at least 33% of the animals by week 5, compared to 3% of the mice at week 5 in the benzo[a]pyrene-only group.

In a short-term assay, Stenius et al. (1989) found that phenol did not increase the production of gamma-glutamyl transpeptidase (GGT) positive foci. Groups of 7-9 male Sprague Dawley rats were partially hepatectomized, treated with diethylnitrosamine, and then with 100 mg/kg phenol by gavage for 5 days (gavage volume not reported). Phenobarbital, the positive control, produced a marked increase in GGT-positive foci. This assay is based on the assumption that GGT-positive cells in enzyme-altered foci represent initiated cells, and the observation that these cells are often resistant to toxicity.

A decrease in tumor formation was seen in a co-carcinogenesis study of phenol and benzo[a]pyrene (Van Duuren and Goldschmidt, 1976; Van Duuren et al., 1971, 1973). Phenol was applied at a dose of 3 mg/application in acetone clipped skin of female ICR/Ha Swiss mice (50/group) three times/week for one year, simultaneously with 5 g of benzo[a]pyrene. The resulting number of tumors (both for papillomas and squamous cell carcinomas) was markedly lower than in the animals receiving the benzo[a]pyrene alone. Phenol alone did not cause skin tumors. Neither the application volume nor application surface area were reported, and no information was provided on any skin effects other than tumors.

In a test of a (TG •AC) transgenic mouse line carrying a v-Ha-ras gene fused to a globin promoter, Spalding et al. (1993) found that phenol did not produce papillomas. This strain has genetically initiated skin and has been shown to be sensitive to the known promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Five male mice were dermally treated twice per week for 20 weeks with 3 mg phenol. Only 1 papilloma was observed, in contrast to strongly promoting agents, which produced 5 or more papillomas per mouse. This dose did, however, cause chronic irritation and hair loss.

A number of studies have investigated why orally administered phenol is not carcinogenic but benzene is, when phenol and many of its metabolites are significant products of benzene metabolism. Medinsky et al. (1995) noted that, based on the urinary metabolite profile, higher levels of hydroquinone are produced after benzene exposure than after exposure to comparable doses of phenol. The potential production of other toxic metabolites, such as muconaldehyde, following benzene exposure, but not phenol exposure, was also noted. The authors explained the different metabolite profiles of phenol and benzene using the zonal distribution of metabolizing enzymes in the liver. As described in Section 3.3, phenol is conjugated in the gut and in zone 1 of the liver. This reduces the amount of phenol that reaches zone 3 of the liver, where oxidative activity is highest, and so decreases hydroquinone production. By contrast, conjugation of benzene in the gut and zone 1 is low, because benzene must be oxidized prior to conjugation. This results in more free phenol reaching zone 3 of the liver following benzene exposure than after phenol exposure, and hence more production of hydroquinone.

Equivocal or negative results were obtained with phenol in a well-conducted and well-controlled interlaboratory study evaluating the usefulness of the Chinese hamster V79 cell metabolic cooperation assay for detecting tumor promoters (Bohrman et al., 1988). The study authors noted, however, that the assay was conducted in the absence of exogenous metabolic activation, and V79 cells have low intrinsic metabolic capacity.

Miyagawa et al. (1995) conducted a validation test in male B6C3F1 mice of the *in vivo-in vitro* replicative DNA synthesis test. The test is based on the hypothesis that nongenotoxic carcinogens are likely to increase cell proliferation. Phenol was negative in this assay, which was conducted at 0, 300, and 600 mg/kg administered via oral gavage.

4.4.2 Genotoxicity

The genotoxic potential of phenol appears to depend on the competing processes of activation to a genotoxic form and metabolic inactivation (e.g., via conjugation). Phenol tended to be negative in bacterial gene mutation assays (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983), but was positive or equivocal in mammalian cell gene mutation assays (McGregor et al., 1988a, 1988b; Paschin and Bahitova, 1982; Tsutsui et al., 1997) (Table 8). Phenol tended to induce micronuclei in mice when administered intraperitoneally (i.p.) (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b), but was negative (or positive only at very high doses) when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in *in vitro* micronucleus tests with human lymphocytes (Yager et al., 1990) and Chinese hamster ovary (CHO) cells (Miller et al., 1995), and caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Results from DNA damage assays are inconsistent, but tend to show that phenol can cause sister chromatid exchanges (Erexson et al., 1985; Ivett et al., 1989) or cell transformation (Tsutsui et al., 1997) if it is not metabolically inactivated.

Phenol was negative in a well-conducted assay *Salmonella typhimurium* reverse mutation assay performed up to cytotoxic doses in the presence and absence of varying concentrations of S9 activation, with strains TA1535, TA1537, TA1538, TA98, and TA100 (Pool and Lin, 1982). Phenol was tested in two independent laboratories as part of a large-scale test by the National Toxicology Program (NTP) in *Salmonella* strains TA1535, TA1537, TA98, and TA100 in the presence and absence of S9 activation (Haworth et al., 1983). Both laboratories found that phenol was negative. Rapson et al. (1980) also reported that phenol was negative in a test in strain TA100, although no primary data were presented and it was unclear if sufficiently high doses were tested. A weak positive response was reported with phenol in strain TA98 in the presence of S9, but not in the absence of S9 (Gocke et al., 1981). Other strains were also tested in that assay, but it was unclear what the results were.

Positive or equivocal results have been reported in mammalian cell gene mutation assays. McGregor et al. (1988a, 1988b) reported on a well-conducted mouse lymphoma L5178Y tk+/tk- assay of phenol performed as part of a test of 72 coded chemicals. In the absence of S9, the results were considered questionable/inconclusive in two independent assays, due to the absence of a dose-related trend and increases occurring only in the presence of high cytotoxicity. In the presence of S9, the first test was questionable (no dose-related trend, but statistically significant results at several doses), but a clear positive result was obtained in the confirmatory test. Overall, the study authors concluded that no clear conclusion was possible.

Other authors have also reported positive results in mammalian gene mutation assays. Paschin and Bahitova (1982) found that phenol was mutagenic in an *in vitro* assay for mutagenicity at the HGPRT locus of V79 Chinese hamster cells in the presence of S9 from the livers of phenobarbital-induced mice. Tsutsui et al. (1997) also reported that phenol induced a dose-related increase mutation frequency in Syrian hamster embryo (SHE) cells.

In contrast with these positive results in mammalian cells, a number of authors (Gocke et al., 1981; Sturtevant, 1952; Woodruff et al., 1985) found that phenol was negative in sex-linked recessive lethal (SLRL) assays conducted in *Drosophila* using the feeding and injection routes.

The potential for phenol to induce micronuclei (a measure of clastogenicity) appears to be related to the route of dosing, with generally positive results when phenol is administered i.p., but negative or equivocal results when phenol is administered orally. This route-related difference is likely due to the potential for first-pass detoxification of phenol when it is administered via the oral route, but not when administered i.p. Several authors have suggested that the chromosomal effects of phenol result from phenol interactions with the spindle apparatus (Bulsiewicz, 1977; Yager et al., 1990). No *in vitro* studies of phenol clastogenesis were located.

Phenol was positive in the micronucleus test in male mice at an i.p. dose of 120 mg/kg (Marrazzini et al., 1994). Similarly, Chen and Eastmond (1995a) reported a weak increase in bone marrow micronuclei of male CD-1 mice treated with up to 160 mg/kg-day phenol i.p. for 3 days. By contrast, Barale et al. (1990) found that phenol was negative when administered at i.p. doses up to 160 mg/kg to male CD-1 mice. However, it is unclear whether sufficiently high doses were tested in that study, since no cytotoxicity and no clinical signs of toxicity were reported; the study also did not include a positive control. Gocke et al. (1981) also found that phenol was negative in male and female NMRI mice treated with phenol i.p. at two daily doses of up to 188 mg/kg per dose, although the sample size of tested animals was small. Ciranni et al. (1988b) found that an oral dose of 265 mg/kg phenol caused a slight increase in micronuclei and some myelotoxicity (decreased ratio of polychromatic to normochromatic erythrocytes, PCE/NCE ratio), but i.p. administration of the same dose caused clear increases in micronuclei and stronger myelotoxicity. Gad El-karim et al. (1985) reported that a single oral dose of phenol (250 mg/kg) was negative for micronucleus formation in male CD mice, but did not report the number of animals tested. This difference between the effects of i.p. and oral administration of phenol is also consistent with the metabolic effects of first-pass metabolism mentioned in Section 3.3.

Ciranni et al. (1988a) found that a single gavage dose of 265 mg/kg phenol caused a small, but statistically significant increase in bone marrow micronuclei, accompanied by cytotoxicity, in pregnant female CD-1 mice treated on GD 13. There was no effect on fetal liver. Although no positive control was included, benzene did cause micronuclei in fetal liver, confirming the sensitivity of the assay.

Phenol was positive in an *in vitro* assay for the development of micronuclei in human lymphocytes, in the absence of exogenous metabolic activation, although the dose response was weak (Yager et al., 1990). Miller et al. (1995) also found that phenol was positive in an *in vitro* micronucleus test in Chinese hamster cells (CHO cells), in the presence or absence of S9 from livers of phenobarbital/beta-naphthoflavone induced rats, although a stronger response was observed in the presence of S9.

Phenol was evaluated in the chromosome aberration assay in CHO cells as part of a series of tests by the NTP to evaluate genotoxicity assays (Ivett et al., 1989). No significant increase was

observed in the absence of S9 activation. In the presence of S9, significant increases in both simple and complex aberrations were observed. A delayed harvest time was used, due to cell cycle delay.

In a five-generation study of chromosome aberrations in spermatogonia and spermatocytes in Porton strain inbred mice, Bulsiewicz (1977) observed dose-related increases in aberrations that tended to increase with successive generations. Polyploidy was also observed. Three dose groups and a control were treated by oral gavage. The dosing volume was reported as "2 mL of a solution of phenol" (sic) for the low-dose group; volumes were not reported for the other groups. Phenol was reported as being administered in a concentration of 0, 0.08 or 0.8 mg/L per day, or 8 g per liter (sic).

A number of studies reported synergistic effects between phenol and hydroquinone in the micronucleus assay in mice (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a). At least part of this interaction is likely due to phenol enhancing the peroxidase-dependent metabolic activation of hydroquinone.

A number of studies have evaluated the potential for phenol to cause DNA damage. These studies tend to show that phenol can produce effects when it is metabolized to an active form, but that inactivation is likely to predominate over activation following oral dosing.

Jansson et al. (1986) found no effect on SCEs in an *in vitro* assay with human lymphocytes, although it was unclear whether sufficiently high doses were tested. Small but statistically significant increases in SCEs in cultured human lymphocytes were reported by Morimoto et al. (1983) and Morimoto and Wolff (1980). By contrast, Erexson et al. (1985) found a dose-related increase in SCEs in human lymphocytes exposed to phenol *in vitro*. Erexson et al. (1985) attributed the difference between their results and the studies that found negative results to differences in the procedure used. In particular, Erexson and colleagues used mitogenic stimulation of the lymphocytes 24 hours prior to the phenol exposure. This mean that the cells were blast transformed and exposed in the G1-S phase (and so there was less opportunity for repair prior to DNA replication), and cytochrome P450 activity was elevated as a result of the blast transformation. Negative controls showed that the increases were not due to the mitogenic stimulation alone. As part of a series of tests by the NTP to evaluate genotoxicity assays, phenol was tested for the induction of SCEs in CHO cells (Ivett et al., 1989). Phenol was positive in the absence of S9, and weakly positive in the presence of S9. Cell cycle delay was observed at all positive doses.

Tsutsui et al. (1997) reported that phenol induced a slight dose-related increase in transformed colonies in an assay with Syrian hamster embryo (SHE) cells, as well as a dose-related increase in unscheduled DNA synthesis (UDS) in the same cell line.

In an evaluation of effects on germ cells *in vivo*, Skare and Schrotel (1984) found no effect on single strand breaks in testicular cells of Sprague Dawley rats receiving a single i.p. injection of up to 79 mg/kg, or five daily i.p. injections of up to 39.5 mg/kg-day. Phenol also did not induce

single strand breaks in mouse lymphoma L5178YS cells (Pellack-Walker and Blumer, 1986), or in Chinese hamster ovary (CHO) cells in a test up to cytotoxic concentrations (Sze et al., 1996).

Reddy et al. (1990) reported that DNA adducts were produced in cultured rat Zymbal glands orally dosed either 750 g/mL of phenol or hydroquinone. The adducts were not chemically characterized and their intensities were not quantified, but no spots were observed autoradiographically in the untreated controls. By contrast, many different adducts were seen in the tissues (bone marrow, Zymbal gland, liver, spleen) analyzed from both untreated female Sprague-Dawley rats and from rats treated for 4 days by oral gavage with a dose of 75 mg/kg-day phenol or 150 mg/kg-day of a 1:1 mixture of phenol and hydroquinone. The adduct patterns and levels of adducts did not differ significantly between control and treated animals. The authors noted that endogenous adducts would interfere with the determination of treatment-induced adducts that chromatograph similarly. To address this possibility, they compared the chromatograms resulting from *in vitro* and *in vivo* treatments. The absence of the major *in vitro* adducts of hydroquinone or benzoquinone in the *in vivo* samples suggested that these adducts were not formed in the whole animal. Conversely, the primary adduct of phenol formed *in vitro* was also observed *in vivo,* although the levels relative to controls were much higher under *in vitro* conditions. The authors suggested that the higher level of adducts following *in vitro* treatment versus *in vivo* treatment could be attributed to detoxification of orally-administered phenol, but did not further address the possibility that there may be a significant basal load of adducts formed by endogenously produced phenol.

Using a fluorescence *in situ* hybridization (FISH) approach, Chen and Eastmond (1995a) found that treatment with phenol alone resulted in micronuclei and breaks in euchromatin, while hydroquinone affected chromosome loss and chromosomal breakage, particularly in centromeric heterochromatin. They suggested that the different pattern of effects with phenol and hydroquinone indicates that the synergism between phenol and hydroquinone is not due solely to phenol-induced increases in hydroquinone metabolism. Instead, the authors suggested that phenol or its metabolites may also be inhibiting DNA repair. In a follow-up study, Chen and Eastmond (1995b) found that phenol alone did not affect the DNA repair enzymes topoisomerase I or topoisomerase II *in vitro*. However, mixing phenol with horseradish peroxidase to mimic the peroxidase metabolism of the bone marrow resulted in complete inhibition of topoisomerase II; no effect was seen when glutathione was added to the peroxidase mixture. In an assay with NCTC 929 mouse fibroblast cells, Yang and Duerksen-Hughes (1998) found that phenol caused a dose-related increase in levels of the p53 protein. The authors noted that cells increase p53 levels in response to DNA damage.

4.4.3 Neurological Effects

As described above, tremors have been observed following relatively high exposures to phenol via the oral (Dow Chemical Co., 1994; Moser et al., 1995) or inhalation (Dalin and Kristoffersson, 1974) routes. Decreased motor activity and a statistically significant increase in rearing post-exposure were also reported in a screening study with rats (Moser et al., 1995), and altered balance was reported in rats exposed continuously via inhalation for 15 days (Dalin and

Kristoffersson, 1974). However, in a 13-week drinking water neurotoxicity study that included extensive neurohistological analyses (ClinTrials BioResearch Ltd., 1998), the only observed effects were decreased motor activity and decreased body weight (compared to the controls), which were probably secondary to decreased water consumption as a result of poor palatability. Based on results of a short-term screening study, neurotoxic effects do not occur at lower exposures than other systemic effects of phenol (Berman et al., 1995; Moser et al., 1995).

4.4.4 Immunotoxicity

As described in Section 4.2, Hsieh et al. (1992) reported immune effects in CD-1 mice administered phenol in drinking water for 28 days. The reported effects include decreased lymphoproliferative responses and decreased mixed lymphocyte responses. In light of inconsistencies in the study, and the unusually low dose at which effects were seen, the results should be considered preliminary. However, this study raises questions regarding the immunotoxic potential of phenol that should be further investigated. Berman et al. (1995) also reported atrophy of the spleen or thymus of rats gavaged with phenol.

4.4.5 Other Studies

Eastmond et al. (1987) investigated the role of phenol in benzene-induced myelotoxicity. No suppression of bone marrow cellularity was observed in male B6C3F1 mice treated intraperitoneally (i.p.) with doses as high as 150 mg/kg twice daily for 12 days (daily doses up to 300 mg/kg). Only minimal suppression was observed in mice dosed with hydroquinone at up to 100 mg/kg, twice per day. By contrast, marked statistically significant, dose-related suppression was seen in mice treated with 75 mg/kg phenol and 75 mg/kg hydroquinone under the same conditions. In further *in vitro* studies, the authors showed that phenol stimulates the horseradish peroxidase-mediated metabolism of hydroquinone, and hypothesized that similar stimulation of local peroxidases occurs in the bone marrow. The observation of myelotoxicity following benzene treatment, but only minimally or not at all after phenol or hydroquinone treatment, was therefore explained by a more than additive interaction between phenol and hydroquinone.

Corti and Snyder (1998) evaluated gender- and age-specific differences in cytotoxicity of benzene metabolites *in vitro*. Bone marrow cells were harvested from adult unexposed male and female Swiss Webster mice, as well as from pregnant females and from fetal males and females. Cultures of CFU-e (colony forming units-erythroid, an erythroid precursor cell particularly susceptible to benzene toxicity) were prepared, and then exposed to different concentrations of the metabolites. While most of the benzene metabolites caused marked cytotoxicity, only minimal toxicity (0-20% cytolethality) of phenol was observed up to the highest concentration tested (40 µM), compared with nearly 100% cytolethality at the same concentration of catechol or hydroquinone. The effects were strongest in cells isolated from fetal females or from virgin adult females, but the dose-response was inconsistent and it appears that no statistical comparisons with the untreated control of the same life stage were done.

Zamponi et al. (1994) studied the mechanism of phenol-induced cardiac arrhythmia, including ventricular tachycardia. As reported in an abbreviated report, the authors suggested that phenol caused cardiac arrhythmia by blocking batrachotoxin-activated cardiac sodium channels. Testing conditions, including doses tested, were not provided in the abbreviated report.

Bishop et al. (1997) investigated the effect of phenol exposure on total reproductive capacity (TRC) in mice. Groups of 26 female hybrid (SEC x C57BL6) F1 mice were given a single intraperitoneal (ip) injection of 0 (buffer solution) or 350 mg/kg of phenol, and the females were caged individually with an untreated male hybrid (C3H/R1 x C57BL10) F1 mouse following the day of injection for 347 days. The animals were observed daily for producing newborn mice, and the young mice were counted and discarded immediately after birth. Female reproductive performance was evaluated based on the total number of offspring per female and the average number of litters per female. The numbers of offspring per female and litters per female in phenol treated mice were comparable to that in the controls. Thus, phenol had no measurable detrimental effect on the parameters used for evaluating long-term reproductive effects in this study.

4.5 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action

Studies investigating the effects of orally administered phenol are summarized in Table 2. When phenol is administered in drinking water, the most common effect is decreased water consumption, presumably due to poor palatability. Effects seen concurrently with decreased water consumption, and probably secondary to dehydration, include body weights lower than those of the controls, decreased maternal body weight compared to controls in developmental toxicity studies, and decreased pup survival prior to culling. Decreased motor activity was also seen in a drinking water neurotoxicity study, but does not appear to be secondary to the decreased water consumption.

Oral exposure to phenol also affects the kidney and liver. Kidney inflammation was observed in the chronic drinking water study in rats (NCI, 1980). Liver and kidney pathology (tubular degeneration, kidney necrosis, and vacuolar degeneration) have also been observed in short-term and subchronic toxicity studies in rats conducted using gavage dosing (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945).

A number of nervous system effects have been observed following phenol dosing. Tremors were observed in one animal that later died (apparently of dehydration) following dosing in drinking water (ClinTrials BioResearch Ltd., 1998). Tremors have also been observed in several gavage studies in rats and mice (NTP, 1983a, 1983b; Dow Chemical Co., 1994; Moser et al., 1995). However, in a specialized 13-week neurotoxicity study in male and female rats that included an FOB and a detailed neurohistopathology evaluation, (ClinTrials BioResearch Ltd., 1998), the only observed nervous system effects were tremors in one animal and decreased motor activity in females. A short-term gavage screening study (Moser et al., 1995) found that the only effect observed in an FOB was a marginal decrease in motor activity and increased rearing post-exposure.

The data regarding the hematotoxic potential of phenol are conflicting. No hematological effects were observed in rats in a well-conducted 2-generation study (IIT Research Institute, 1999). By contrast, decreased hematocrit and erythrocyte counts were seen at much lower doses in a 28-day drinking water study in mice (Hsieh et al., 1992). Data from these two studies are contrasted in Table 5. It is unclear if the differences between these studies reflect interspecies differences, or whether issues regarding the study design affected the observed effect in the mouse study. The chronic drinking water studies conducted by NCI (1980) in mice and rats do not shed light on this issue, because no hematological evaluation was conducted. However, the overall database on phenol do not support the conclusion that mice are more than 50-fold more sensitive than rats for hematological effects (NOAEL of 6 mg/kg-day in mice versus free-standing NOAEL of 301 mg/kg-day for hematological effects in the 2-generation rat study). The general systemic toxicity data indicate that the toxicity of phenol in rats and mice is generally similar. For example, the NOAELs in the NCI (1980) two-year drinking water studies for rats and mice were 260 mg/kg-day and 450 mg/kg-day, respectively. Similarly, the NOAELs from the NCI (1980) 90-day drinking water studies in rats and mice conducted in order to set doses for the two-year studies were 480 mg/kg-day and 450 mg/kg-day for rats and mice, respectively. The oral LD_{50}s for rats and mice are also within a factor of two, as described in Section 4.2. The similarity of these systemic NOAELs and LD_{50}s indicate that tissue levels of the toxic agent (phenol or a metabolite) are similar in mice and rats. Although there could be differences in the sensitivity of bone marrow or blood cells of mice and rats to phenol, such differences are unlikely to be of the size that can explain the inconsistency between the results of the Hsieh et al. (1992) and IIT Research Institute (1999) studies. Therefore, the results of the Hsieh et al. (1992) study are considered suspect in the absence of a confirmatory study in mice.

The Hsieh et al. (1992) study also reported immunotoxic effects in a number of assays of splenocyte preparations of phenol-exposed mice, although the results of this study should be considered preliminary, due to inconsistencies across endpoints. “Necrosis or atrophy in the spleen or thymus” (not further described) was observed in a 14-day screening study of rats gavaged with phenol (Berman et al., 1995), supporting the immune organs as targets of phenol. Baj et al. (1994) reported in an epidemiology study of Polish workers that exposure to Ksylamit® vapor resulted in immune effects, but it is unclear if phenol is the causative agent, since Ksylamit® contains a number of different aromatic compounds.

Benzene, which is metabolized to phenol, among other compounds, causes immunological effects, including lymphopenia and leukopenia (reviewed in ATSDR, 1998). However, although benzene is a leukemogen in humans, it has not been shown to induce leukemias in experimental animals. For example, in the NTP gavage studies of benzene (NTP, 1986), it was carcinogenic to both male and female Fischer 344 rats and $B6C3F_1$ mice, inducing tumors at multiple sites. There was a statistically significant increase in lymphomas in male and female mice, but not in rats of either sex. No significant increase in leukemias was noted in either species.

One proposed mechanism by which this immunotoxicity is induced involves the interaction between phenol and hydroquinone, in which benzene stimulates the metabolism of hydroquinone. Eastmond et al. (1987) observed decreased bone marrow cellularity in male mice dosed i.p. with

phenol and hydroquinone, but not with phenol alone at doses up to 300 mg/kg-day for 12 days, and only minimally with hydroquinone. These results appear to contradict the results reported by Hsieh et al. (1992) at much lower doses in mice, although the target tissue examined was bone marrow in the Eastmond et al. (1987) study, compared with spleen in the Hsieh et al. (1992) study. The former study was also conducted via the i.p., rather than oral, route, but toxicity might be expected to be higher via the i.p. route, since first-pass metabolism would be lower.

It is not known with certainty whether the toxic effects of phenol are due to the parent compound or its metabolites. Distinguishing between effects of the parent compound and its oxidative metabolites is complicated by the lack of adequate data on concurrent blood levels of parent compound and metabolites. Phenol and metabolite levels in blood would be expected to rise in parallel as portal of entry metabolism becomes saturated. However, several lines of reasoning suggest that many of the toxic effects are due to the oxidative metabolites of phenol. In an *in vitro* study of dysmorphogenic and embryotoxic effects on whole rat conceptuses of benzene, phenol, and their metabolites, Chapman et al. (1994) found that phenol toxicity was much higher in the presence of S9. The target tissues of phenol toxicity (kidney, liver, lung, and possibly bone marrow) are also those in which phenol can be oxidatively metabolized. In a 2-week inhalation study, Dalin and Kristoffersson (1974) observed altered balance and twitches in the absence of increased amounts phenols in blood, suggesting that a phenol metabolite, rather than phenol itself, is the toxic agent. Alternatively, the analytical method used may not have been sensitive enough or specific enough to detect any changes in blood levels of phenol. Phenol could produce portal of entry and systemic toxicity as a result of its ability to react with, and denature, protein.

A key point with regard to the evaluation of the toxicity of orally-administered phenol is whether gavage studies accurately represent the toxicity under environmental exposure conditions. Gavage studies are typically done using a single bolus dose per day, while environmental exposure is more likely to involve exposure distributed over the course of the day, similar to dosing in the animal drinking water studies. Figure 2 compares the doses and observed severity of effects in drinking water and gavage studies. Of particular interest is the number of gavage studies in which death, a frank effect, was observed, while drinking water studies at comparable or higher daily doses produced only tremors, kidney inflammation, and effects secondary to decreased water intake. The gavage NOAELs that occur at the same doses as gavage adverse effect levels (AELs) are for maternal and developmental toxicity. The sole exception to the large difference between gavage and drinking water studies in doses that cause effects is the 28-day drinking water study of Hsieh et al. (1992), which reported hematological and immune effects at doses comparable to NOAELs in drinking water studies. As noted above, there are a number of questions regarding the interpretation of the Hsieh et al. (1992) study.

Toxicokinetic data support this difference between gavage and drinking water studies, and indicate that toxicity is correlated with peak blood concentrations, rather than the area under the curve. Dow Chemical Co. (1994) observed “phenol twitching behavior” (including tremors and eye blinking) in rats gavaged with 150 mg/kg phenol, and the behavior disappeared in less than an hour post-dosing, as phenol blood levels declined below peak values. By contrast, no twitching behavior was observed following a similar daily dose of phenol in drinking water. Unfortunately,

blood levels of phenol or its metabolites were not determined in the drinking water phase of the study, but they are likely much lower than in the gavage phase, in light of the rapid blood clearance. The higher systemic toxicity of gavaged phenol when it is administered in smaller volumes (NTP, 1983a) also supports the idea that toxicity is related to peak blood concentrations, because smaller dosing volumes would be expected to enhance the absorption rate. An unpublished GLP range-finding study for maternal toxicity (International Research and Development Corporation 1993) also found higher systemic toxicity for phenol when it was administered in smaller dosing volumes.[9] Data on the relationship between dosing volume and peak phenol blood concentrations are not available. Data on the relationship between peak blood concentrations and effects are also not available for the endpoints relevant to the critical effect.

The inhalation data on the effects of phenol are very limited (Table 6). Only one study (Huntingdon, 1998) conducted according to modern toxicological methods was located, and the exposures in this study were for only 2 weeks. Other studies ranged from 2 weeks (Dalin and Kristoffersson, 1974) to 90 days (Deichmann et al., 1944; Sandage, 1961), but they included incomplete documentation of the study results, and did not use modern methods for controlling exposure levels. In addition, the authors of some of the studies (e.g., Sandage, 1961) appear to have been looking for marked effects, and appear to have dismissed statistically significant incidences of organ pathology of lesser severity. Nonetheless, the studies are fairly consistent with regard to the target organs and effects observed. Exposure to high concentrations produced nervous system effects, while liver, kidney, and lung pathology occurred at lower concentrations. Rats were reported to be much less sensitive than rabbits or guinea pigs (Deichmann et al., 1944). The systemic targets observed following inhalation exposure to phenol are supported by data from the oral exposure route.

Information on the mode of action of inhaled phenol toxicity is also quite limited, but some extrapolation from other routes is possible. Based on the irritative and corrosive effects seen following dermal exposure to phenol, the respiratory tract effects of phenol are likely due to direct contact of phenol with the respiratory tract tissue. As noted in Section 3, phenol is extensively absorbed following inhalation exposure. The lung can metabolize phenol prior to absorption, but the efficiency of metabolism in the lung is lower than that for the gut or liver (Cassidy and Houston, 1984). After the inhaled phenol (and its metabolites) reaches the blood stream, the same points described above for the oral route are relevant. In brief, it is not known whether the toxic effects are due to phenol itself or its metabolite(s), but at least some of the toxic effects appear to be attributable to phenol metabolite(s) (Chapman et al., 1994). Systemic toxicity appears to be related to peak concentrations in blood, rather than the AUC.

There have been no studies specifically investigating the mode of action for the red nasal discharge observed in the Huntington (1998) study, but it appears likely that this effect is a result of the direct contact of phenol with the highly vascularized tissue at the surface of the nasal

[9]Although this unpublished study is not of high quality, and was not peer-reviewed, the study is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

epithelium. This suggestion is supported by the direct contact reactivity of phenol with skin surfaces, and by the absence of associated histopathology.

4.6 Weight of Evidence Evaluation and Cancer Classification - Synthesis of Human, Laboratory Animal and Other Supporting Evidence, Conclusions about Human Carcinogenicity, and Likely Mode of Action

Chronic drinking water bioassays of phenol have been conducted in rats and mice (NCI, 1980). In these studies, the NCI concluded that phenol was "not carcinogenic" in male or female F344 rats or B6C3F1 mice. However, the report also noted that leukemia and lymphoma were statistically significantly increased in low-dose male rats, although there was no significant increase at the high dose. The increases in leukemia are of particular interest, in light of the leukemogenic effects of benzene (for which phenol is a metabolite) in humans. (In experimental animals, benzene has not been shown to induce leukemia, although increases in lymphoma have been observed [e.g., NTP, 1986].) The MTD was clearly reached in the rat study (NCI, 1980), based on decreased body weight compared to controls, and based on kidney histopathology. Although the only sign of toxicity in the mouse study was decreased body weight (compared to the controls) secondary to decreased water consumption, higher doses probably could not have been tested, in light of the decreased water consumption. Higher toxicity probably could have been achieved in a gavage study in mice, at lower doses. These considerations suggest that an MTD was also reached in mice, although a definitive conclusion is difficult. No other long-term oral carcinogenicity studies of phenol are available. No inhalation studies of phenol were of a sufficient duration to assess phenol carcinogenicity. The only long-term study assessing the carcinogenicity of phenol applied dermally without initiation was Boutwell and Bosch (1959), in which increased papillomas were seen at a dose that also caused ulceration.

In contrast with these negative results for oral carcinogenicity, dermally administered phenol has been consistently observed to be a promoter. Several authors (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959; Wynder and Hoffmann, 1961) observed that dermally applied phenol promoted DMBA-initiated skin tumors. These studies have generally reported significant skin ulceration at all phenol doses tested. The exception is Wynder and Hoffman (1961), who reported that 5% phenol promoted DMBA-initiated tumors in mice in the absence of any toxic reactions. When the same phenol dose was administered in different volumes, higher promotion activity was exhibited by the more concentrated solution, which also produced severe skin ulceration, suggesting that some of the promotion activity may have been related to the rapid cell division in repairing of skin damage (Salaman and Glendenning, 1957). The observed response was dose-related (Boutwell and Bosch, 1959), but marked systemic toxicity was also observed at these doses. Co-carcinogenesis with dermally administered benzo[a]pyrene has also been observed (Wynder and Hoffmann, 1961). Because the benzo[a]pyrene was co-administered with the phenol, this assay cannot be classified as a true initiation/promotion assay. Production of papillomas by dermally administered phenol (in the absence of an initiator) was observed only at a concentration that caused ulceration, and hence was above the maximal tolerated dose (MTD).

Genotoxicity studies have found that phenol tends not to be mutagenic in bacteria (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983), but positive or equivocal results have been obtained in gene mutation assays in mammalian cells (McGregor et al., 1988a, 1988b; Paschin and

Bahitova, 1982; Tsutsui et al., 1997). Increases were larger in the presence of S9 activation. Phenol tended to induce micronuclei in mice when administered intraperitoneally (i.p.) (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b), but negative (or positive only at very high doses) when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in *in vitro* micronucleus tests with human lymphocytes (Yager et al., 1990) and CHO cells (Miller et al., 1995), and caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Phenol has been observed to act synergistically with hydroquinone in the production of genotoxic effects (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a).

Epidemiology data do not shed further light on the carcinogenic potential of phenol. Some studies (Kauppinen et al., 1986; Dosemeci et al., 1991) have reported elevated risks in phenol-exposed workers, while others have observed no effect (Wilcosky et al., 1984). However, the usefulness of each of these studies for risk assessment is limited by (depending on the study) an absence of an effect when latency was considered, a lack of a dose-response, and potential for confounding.

While phenol was negative in oral bioassays conducted in rats and mice (NCI, 1980), questions remain regarding the carcinogenic potential of phenol, in light of the positive results in initiation/promotion assays (albeit at exposures typically above the MTD), the increases in leukemia in low-dose male rats in the oral bioassay, and the observation of gene mutations in mammalian cells *in vivo* and micronuclei *in vivo* following i.p. dosing. No inhalation studies of sufficient duration to assess phenol carcinogenicity have been conducted. Dermal carcinogenicity or initiation/promotion studies with phenol at exposures below the MTD have not been conducted. The carcinogenic potential of phenol via inhalation exposure has not been evaluated at all. Under the Proposed Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1996a) the carcinogenicity of phenol via the oral, inhalation, and dermal exposure routes *cannot be determined* due to inadequate data. Under the current guidelines (U.S. EPA, 1987), phenol falls in Category D, not classifiable as to human carcinogenicity.

Similar conclusions have been reached by other groups in recent assessments of the carcinogenicity of phenol. IARC (1999) concluded that there is *inadequate evidence* in humans for the carcinogenicity of phenol, and *inadequate evidence* in experimental animals for the carcinogenicity of phenol. Overall, IARC (1999) concluded that phenol is *not classifiable as to its carcinogenicity to humans (Group 3).* Phenol is not included in the Ninth Report on Carcinogens (NTP, 2000a), and is not listed as being considered for inclusion in the 10th Report on Carcinogens (NTP, 2000b). This report only contains chemicals and substances classified as known human carcinogens or as reasonably anticipated to be carcinogens.

4.7 Susceptible Populations

People with decreased ability to conjugate and eliminate phenol, such as those with low activities of phenol sulfotransferase or glucuronyltransferase, may be more susceptible to phenol toxicity.

4.7.1 Possible Childhood Susceptibility

As discussed in Section 5.1, the co-critical effects for ingestion toxicity of phenol are decreased fetal body weight (NTP, 1983a) and decreased maternal body weight in a developmental toxicity study (Argus Research Laboratories, 1997), supported by decreased motor activity (ClinTrials BioResearch, 1998). As shown in Table 2, the decreased fetal body weight in one of the co-principal studies (NTP, 1983a) occurred at a dose below that at which maternal toxicity occurred, although maternal toxicity occurred at the lower dose in the other co-principal study (Argus Research Laboratories, 1997). Because one of the co-critical effects occurs in the fetus, developing fetuses are identified as a possible susceptible population. The strength of this conclusion is weakened, however, by the small magnitude of the fetal weight increase, together with the increased litter size, which led to the high dose being identified as an *equivocal* LOAEL (see Section 4.3).

Only one study was located that specifically addressed age-related differences in the systemic toxicity of phenol was located. Deichmann and Witherup (1944) compared the lethality of an oral dose of 600 mg/kg phenol (administered as a 5% aqueous solution) in 10-day-old, 5-week-old, and adult rats. Percent mortality was 90%, 30%, and 60% in the neonates, young rats, and adult rats, respectively. While the young and adult rats died within 1.5 hours of dosing, the neonates died 12-24 hours after dosing. The data from this study suggest that neonates are more sensitive than adults, and young rats may be less sensitive than adults. Alternatively, the age-related differences observed by Deichmann and Withrup (1944) could reflect inter-individual variability that was not a consequence of age. Since this study has not been replicated, definitive conclusions are not possible.

Data from humans and rats are consistent in showing very little fetal expression of CYP2E1, which is rapidly induced shortly after birth and rises though childhood (reviewed in Hakkola et al., 1998). The age after parturition at which CYP2E1 levels peak has been studied in laboratory animals with inconsistent results reported. Some studies suggest that peak levels are reached during childhood with a subsequent decrease to adult levels (Schenkman et al., 1989), while others have shown a rapid rise in CYP2E1 levels during childhood to a maximum level that is sustained during adulthood (Song et al., 1986). If the toxic moiety is a metabolite, decreased P450 metabolism could be expected to result in decreased toxicity. However, since sulfate conjugation is also lower early in life (Iwasaki et al., 1993), more of the dose is available for oxidative metabolism. Indeed, Heaton and Renwick (1991) found higher production of oxidative metabolites in young rats. This difference was higher in males, with the percentage of the administered dose recovered as hydroquinone conjugates decreasing from 38% of the administered dose in 3-week-old males to 8.2% in 16-week-old rats. Smaller decreases with age

(from 17.8% of the administered dose in 4-week-old rats to 10.5% in 15-week-old rats) were observed in females. These data suggest the potential for children to be more sensitive than adults to the systemic effects of phenol.

4.7.2 Possible Gender Differences

Kenyon et al. (1995) (in mice) and Heaton and Renwick (1991) (in rats) reported higher excretion of hydroquinone conjugates in males than in females, suggesting higher levels of hydroquinone production in males. By contrast, Meerman et al. (1987) reported only slightly faster metabolism in male rats. These data would tend to suggest that, if hydroquinone is the toxic moiety, phenol would be more toxic in males. However, few differences in phenol toxicity between males and females were identified; differences in NOAELs reflect differences in water consumption per unit weight, resulting in differences in estimated intake. Acute oral lethality data do suggest that phenol is more toxic to males (Thompson and Gibson, 1984).

5. DOSE RESPONSE ASSESSMENTS

5.1 Oral Reference Dose (RfD)

5.1.1 Choice of Principal Study and Critical Effect

An extensive database for the effects of orally administered phenol is available. The studies relevant to the development of the RfD are summarized in Table 2. Two-year drinking water studies conducted in rats and mice are available (NCI, 1980). Hematology and serum biochemical evaluations were not included in those chronic studies, but were included in a recent 2-generation drinking water study conducted in rats (IIT Research Institute, 1999). The only study evaluating hematological effects in mice is a 28-day drinking water study (Hsieh et al., 1992). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch Ltd., 1998). A number of developmental toxicity studies have been conducted in rats and mice, all via the gavage route (Argus Research Laboratories, 1997; NTP, 1983a; NTP, 1983b, Narotsky and Kavlock, 1995).

As shown in Table 2, the study with the lowest NOAEL/LOAEL boundary is the 28-day drinking water study in mice of Hsieh et al. (1992). In this study, the NOAEL was 6.3 mg/kg-day, and the LOAEL was 33.6 mg/kg-day, based on decreased hematocrit and decreased immune parameters at the high dose. Statistically significant decreases in erythrocyte counts were observed at the low and mid doses, but were not considered biologically meaningful for the purposes of this assessment, in the absence of effects on hematocrit and in light of the lack of support from other studies. As noted in Section 4.2, there are a number of uncertainties regarding the reliability of this study, in light of inconsistencies in the immune parameter results, and in light of the absence of any hematology effects at much higher doses in the 2-generation rat study (IIT Research Institute, 1999). The available toxicity data do not support the differences in general phenol toxicity of more than 50-fold that would be needed to reconcile these two studies. The absence of effects on bone marrow cellularity in mice at phenol doses up to 300 mg/kg-day

administered i.p. for 12 days (Eastmond et al., 1987) also raises questions about the effects results of Hsieh et al. (1992). Similarly, Corti and Snyder (1998) evaluated the effects of benzene metabolites on CFU-e cells (an erythroid progenitor cell sensitive to benzene) harvested from mice, and found that the cytotoxicity of phenol was much lower than that of other benzene metabolites. Effect in mice and rats were not compared in that study. In light of these questions, and in the absence of data supporting the observed effects at such low doses, the results of Hsieh et al. (1992) are too preliminary to be used as the basis for the phenol RfD. However, this study does raise concerns regarding the potential of phenol to cause hematological and immune effects, and it would be worthwhile to address these questions in a longer-term mouse study. The uncertainties regarding these endpoints are further addressed in Section 5.1.3.

The next lowest NOAEL/LOAEL combination was observed in a 14-day gavage study in rats conducted as part of a large-scale screening study of a number of chemicals (Berman et al., 1995; Moser et al., 1995). Tremor, kidney tubular degeneration, and increased rearing in an FOB were observed at the high dose of 40 mg/kg-day, but not at the next lower dose of 12 mg/kg-day. Although the incidence of kidney histopathology was not statistically significant, the high dose can be considered a LOAEL, in light of the low statistical power of the study (only 8 female rats/group), and the rarity of these lesions in short-term studies. The corresponding NOAEL is 12 mg/kg-day. The relevance of this NOAEL to environmental exposures is questionable, however, due to the markedly higher toxicity observed in gavage studies than in drinking water studies, as discussed in Section 4.5, and the absence of supporting toxicity in drinking water studies of much longer duration. In particular, drinking water studies found no kidney histopathology in rats exposed to 260 mg/kg-day for 2 years (NCI, 1980) (although kidney inflammation was observed at higher doses), in mice exposed to doses up to 660 mg/kg-day for 2 years (NCI, 1980), or in parental rats in a 2-generation reproduction study (IIT Research Institute, 1999). The only other study reporting kidney histopathology at low doses was a poorly documented and unpublished 1945 6-month gavage study in rats (Dow Chemical Co., 1945). The very small dosing volume used by Berman et al. (1995) also may have contributed to the high toxicity, in light of the findings of NTP (1983a).

Two co-principal studies are the most appropriate for the derivation of the RfD. NTP (1983a) found decreased fetal weight in CD rats gavaged on GD 6-15 with 120 mg/kg-day. The high dose of 120 mg/kg-day was considered an *equivocal* LOAEL for developmental effects, in light of the small magnitude of the weight decrease and the increased litter size. The NOAEL for developmental toxicity was 60 mg/kg-day, and the maternal toxicity NOAEL was the high dose, 120 mg/kg-day. Argus Research Laboratories (1997) found decreased maternal weight gain in rats gavaged on gestation day 6-15 with 120 mg/kg-day phenol; the maternal NOAEL was 60 mg/kg-day based on decreased body weight gain, and the developmental NOAEL was 120 mg/kg-day. Although both of these studies were conducted via the gavage route, it is less clear that the developmental toxicity of phenol administered via gavage is markedly different from that of phenol in drinking water. The same NOAEL (60 mg/kg-day) was identified using a standard gavage protocol (NTP, 1983a) and using a protocol in which the daily dose was divided into three administrations per day (Argus Research Laboratories, 1997), although the former was for developmental toxicity and the latter for maternal toxicity. Data on the placental transfer of phenol

or its metabolites are extremely limited, and so it is unclear how the fetal dose relates to peak or total blood phenol levels. The study with the divided gavage dosing protocol (which would presumably decrease the peak blood levels) both support and question the hypothesis that fetal toxicity is higher following gavage dosing than following continuous exposure The Argus Research Laboratories (1997) study had a lower maternal NOAEL, but a higher developmental NOAEL, than that seen in the NTP (1983a) study. Between the two studies, there is the consistent result that 60 mg/kg-day is a NOAEL, and effects on body weight compared to controls begin to occur at 120 mg/kg-day.

Although the decreased fetal body weight (NTP, 1983a) and decreased maternal weight gain (Argus Research Laboratories, 1997) were mild effects and possibly confounded by the gavage dosing, these results are supported by a drinking water study. Decreased motor activity was seen in female rats consuming the high concentration of phenol (5000 ppm, corresponding to 360 mg/kg-day) in the 13-week neurotoxicity study (ClinTrials BioResearch, 1998). The NOAEL in females was 107 mg/kg-day. As discussed in Section 4.2, the study authors considered the decreased motor activity to be secondary to dehydration, but analysis of the individual animal data and comparison with the literature could not confirm this assumption. IIT Research Institute (1999) conducted a 2-generation drinking water study in rats in which decreased parental and pup weight occurred at a LOAEL of 301 mg/kg-day, with a NOAEL of 71 mg/kg-day. However, these lower body weights compared to control are likely to be secondary to decreased water consumption.

5.1.2 Method of Analysis

The NOAEL of 60 mg/kg-day for decreased fetal body weight (NTP, 1983a) and decreased maternal weight gain (Argus Research Laboratories, 1997) was used as the basis for the RfD. Benchmark dose (BMD) modeling was not conducted on the fetal weight endpoint, because fetal weights were reported only as an average across both sexes for all litters; no individual animal data were available. Because fetal weights of male fetuses tend to be heavier than females, and because the number of fetuses per litter affects the fetus weight, it was not appropriate to model the pooled data. BMD modeling could be done on the maternal weight gain endpoint.

5.1.3 RfD Derivation

The data on the within-human variability in the toxicokinetics and toxicodynamics of ingested phenol are insufficient to adjust the default uncertainty factor for intraspecies variability. In a sample of liver fractions from 10 people, Seaton et al. (1995) found that the kinetics of phenol sulfation and hydroquinone conjugation varied by up to approximately 3-fold. Much larger variability in CYP2E1 is found, particularly between neonates and adults (Vieira et al., 1996). These data on inter-individual variability in enzymatic metabolism are not adequate to move from the default UF_H of 10, because they do not reflect potential variability in portal-of-entry metabolism of phenol, or uncertainty regarding the identity of the toxic moiety.

The absorption, distribution, and metabolism of ingested phenol in rats and humans appear

to be generally quantitatively similar, although the data are insufficient for a quantitative comparison. Comparison of laboratory animal and human phenol toxicokinetics is also limited by the lack of knowledge regarding the identity of the toxic moiety. It is not possible to quantitatively use the toxicokinetic data to adjust the default 10-fold factor for interspecies variability, and the default UF_A of 10 is judged to be appropriate. It may be possible to reduce this default value of 10, however, if future data (perhaps supplemented by a PBPK model) comparing the toxicokinetics of phenol and its metabolites in the placenta and fetus of rats and humans become available.

Because a NOAEL was used, no UF is required for extrapolation from a NOAEL to a LOAEL. Similarly, no UF for extrapolation across duration is needed, since this developmental study is supported by chronic bioassays in two species.

The oral database for phenol can be considered complete. It includes 2-year drinking water studies conducted in rats and mice (NCI, 1980), a 2-generation drinking water study conducted in rats (IIT Research Institute, 1999), and a gavage developmental toxicity studies in rats (Argus Research Laboratories, 1997; NTP, 1983a; Narotsky and Kavlock, 1995) and mice (NTP, 1983b). However, the range of endpoints evaluated in the chronic toxicity studies was limited, and did not include hematological or serum biochemistry evaluations. Hematological effects in mice were observed by Hsieh et al. (1992) in a 28-day drinking water at low doses. By contrast, these endpoints were evaluated, and no significant hematological or serum biochemistry effects were observed, at doses up to more than 300 mg/kg-day in the two-generation rat study (IIT Research Institute, 1999). An i.p. study of the effects of phenol on bone marrow cellularity in mice at doses up to 300 mg/kg-day (Eastmond et al., 1987) and an *in vitro* study with mouse bone marrow cells (Corti and Snyder, 1998) also do not indicate that mouse blood cells are highly susceptible to effects of phenol. Thus, while it would be useful to conduct a long-term mouse study that evaluates hematological effects, an additional uncertainty factor (i.e., a database uncertainty factor) to address this data gap is not needed. An additional degree of public health protection is provided by the use of a gavage study, rather than the more environmentally relevant route of drinking water.

A composite UF of 100 results. No MF is applied, because the existing uncertainties have been addressed with the standard uncertainty factors.

RfD = 60 mg/kg-day /100 = 0.6 mg/kg-day, or 6E-1 mg/kg-day.

Note that this RfD is applied to ingested phenol *in addition to* the normal daily endogenous production of phenol.

An additional uncertainty factor for sensitive populations such as infants and children is not needed for phenol, because sufficient studies of reproductive and developmental toxicity have been performed, and the endpoint used for the oral RfD is decreased fetal body weight in a developmental toxicity study in which no terata were observed.

5.2 Inhalation Reference Concentration (RfC)

5.2.1 Choice of Principal Study and Critical Effect

The minimal database needed for the development of a RfC is a well-conducted subchronic inhalation study that adequately evaluated a comprehensive array of endpoints, including the respiratory tract, and established a NOAEL and LOAEL (U.S. EPA, 1994b). This criterion was not met for phenol. Neither of the two available subchronic studies (Deichmann et al., 1944; Sandage, 1961) are adequate for exposure-response assessment, because neither included adequate documentation of the histopathology results, and neither used modern methods for generating or monitoring exposure levels. These studies can, however, be used for hazard identification, and identify the respiratory tract, liver, and kidney as targets of inhalation exposure to phenol. The phenol database also includes a well-conducted 2-week inhalation study with rats that used modern exposure methods, evaluated a wide array of endpoints, and included a thorough histopathology evaluation of the respiratory tract (Huntingdon, 1998). This study is unpublished, but it was conducted according to GLP guidelines, and so included more thorough documentation and quality control than many published studies. The only treatment-related effect observed was a red nasal discharge in male rats, which was observed with a statistically significant, duration-related and concentration-related incidence in the mid- and high-concentration groups.

In the absence of an inhalation study of sufficient duration, no RfC for phenol can be derived. A route-to-route extrapolation is not appropriate, since phenol can be a direct contact irritant, and so portal of entry effects are a potential concern.

5.2.2 Methods of Analysis

No RfC was derived.

5.2.3 RfC Derivation

No RfC could be derived, due to insufficiencies of the database.

5.3 Cancer Assessment

As discussed in Section 4.6, the carcinogenicity of phenol *cannot be determined*. Phenol was negative in oral carcinogenicity studies in rats and mice, but questions remain regarding increased leukemia in male rats in the bioassay, as well as the positive gene mutation data and the positive results in dermal initiation/promotion studies at doses at or above the MTD. No inhalation studies of an appropriate duration exist. Therefore, no quantitative assessment of carcinogenic potential via any route is possible.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1 Hazard Identification

6.1.1 Oral Noncancer

Phenol is used in a number of industrial products, as well as in over-the-counter medicines, such as cough drops and mouthwashes. Use of these consumer products can result in short-term high-level phenol exposures. ATSDR (1998) estimated that intake of the maximum recommended dosage of 300 mg phenol/day would result in an approximate dose of 4-8 mg/kg-day. Use of these products on a daily basis over the course of a lifetime would result in a dose higher than the RfD derived in this document, but these products are not intended for use over a prolonged period. In addition, the maximum recommended dosage may not be protective of pregnant women and fetuses, as consumers are advised "as with any drug, if you are pregnant or nursing a baby, seek the advice of a medical professional before using this drug."

In most studies of phenol administered in drinking water, water consumption was markedly decreased at the highest dose, presumably due to poor palatability. A number of toxic effects secondary to the decreased water consumption have been observed, including decreased body weight compared to controls, decreased pup weight, and decreased pup survival pre-culling. Other effects that may not have been secondary to decreased water consumption were kidney inflammation (NCI, 1980) and decreased motor activity (ClinTrials BioResearch Ltd., 1998). Gavage studies found more severe effects and saw these effects at lower doses. Observed effects included lung, liver, and kidney pathology; tremors and other nervous system effects; and, at sufficiently high doses, death. These data suggest that the toxicity of phenol is higher via gavage dosing than via administration in drinking water. The suggestion is supported by the finding that a series of behaviors termed "phenol twitching behavior" correlate with peak blood levels, rather than area under the curve (Dow Chemical Co., 1994). For a given daily dose, peak blood levels would be much higher following gavage dosing than following continuous administration in water. A direct comparison of the toxicity of phenol when administered via these two routes could determine definitively whether route-specific differences exist. Nonetheless, the data supporting the higher toxicity of phenol administered by gavage was considered sufficiently strong that it was not considered appropriate to use the Berman et al. (1995) study (which also used small dosing volumes) as the principal study.

Developmental toxicity studies have been conducted only via the gavage route. The only sign of developmental toxicity observed in these studies was decreased fetal body weight. In support of the effect in the principal studies, decreased fetal body weight compared to controls was observed in the companion study in mice (NTP, 1983b), although this was in the presence of significant maternal toxicity. In the absence of developmental toxicity studies for phenol using the drinking water route, and in the absence of data on the rate of phenol transfer across the placenta, the data were insufficient to exclude the use of a developmental toxicity study in which phenol was administered via gavage. In addition, the same NOAEL was identified when the same daily doses were administered using a divided dosing protocol of three doses/day, although this NOAEL was based on decreased maternal weight gain (Argus Research Laboratories, 1997). Therefore, the rat gavage studies by NTP (1983a) and Argus Research Laboratories (1997) were co-principal studies. The co-critical effects of decreased fetal body weight and decreased maternal weight gain in rats were used to calculate the RfD. It should be noted, however, that the next higher dose in the NTP (1983a) study was an *equivocal* LOAEL, because the observed decrease in fetal weight was small (but statistically significant) and increased litter size was also seen at this dose. It is possible, therefore, that the dams were near the limit of what they could carry in terms of pup burden (total fetal weight).

The NOAEL was supported quantitatively by the NOAEL of 107 mg/kg-day for decreased motor activity in a 90-day drinking water neurotoxicity study (ClinTrials BioResarch Ltd., 1998). A NOAEL of 71 mg/kg-day for decreased parental and pup body weights was also identified in a drinking water two-generation reproduction study (IIT Research Institute, 1999), although these effects are likely secondary to decreased water consumption.

A key uncertainty in the development of the RfD is the interpretation of the study by Hsieh et al. (1992). Hematotoxicity (decreased hematocrit) and immunotoxicity (decreased lymphocyte proliferation and decreased mixed lymphocyte reaction) were observed in this 28-day drinking water study in mice at doses much lower than the doses than produced toxicity in other studies. However, study inconsistencies raise questions regarding the reliability of these results. In addition, no hematotoxic effects were seen at much higher doses in a two-generation drinking water study in rats (IIT Research Institute, 1999). It is unclear whether these differences in study results reflect interspecies differences between rats and mice, or whether one of the studies produced spurious results. However, the absence of effects on bone marrow cellularity in mice at daily phenol doses up to 300 mg/kg-day administered i.p. for 12 days (Eastmond et al., 1987), and the minimal effects seen in an *in vitro* study with CFU-e mouse bone marrow cells (Corti and Snyder, 1998) also raise questions about the results of Hsieh et al. (1992). In addition, the general similarity of systemic phenol toxicity in rats and mice argues against significant differences in metabolism that could lead a large interspecies difference in phenol toxicity. The immunotoxicity data are too preliminary to be used as the basis for a risk assessment, because of questions regarding the consistency of the data and the clinical significance of the results. It would be useful for other laboratories to conduct additional studies to determine whether the results of Hsieh et al. (1992) can be replicated.

Although not directly affecting the determination of the RfD, uncertainty also exists

regarding whether the decreased motor activity in females reported by ClinTrials BioResearch Ltd., (1998) was due to dehydration only, or whether phenol exposure also contributed to the effect. The NOAEL from this study was used as supporting data for the principal study. The study authors considered the decreased motor activity to be attributable to dehydration, based on the marked decrease in water intake, and on the absence of supporting changes in the FOB. By contrast, this assessment concluded that phenol at least contributed to the effect, since there was no clear correlation between individual animals with dehydration and with decreased activity, and because the limited literature on the topic report no effect on motor activity of water deprivation for several days. A neurotoxicity study in which the controls were allowed only limited access to drinking water would also address this issue.

6.1.2 Inhalation Noncancer

The database for inhalation toxicity of phenol is very limited. A well-conducted 2-week study is available (Huntingdon, 1998), but the duration is less than that appropriate for serving as the basis for the RfC. Longer-term studies have been conducted (Deichmann et al., 1944; Sandage, 1961), but are limited by inadequate control of exposure levels, unclear sensitivity of the evaluation, and limited reporting.

The inhalation toxicity studies are sufficient however, to identify the respiratory tract, liver, kidney, and nervous system as targets of inhaled phenol toxicity. A significant uncertainty is which species is the most appropriate for extrapolation to humans. Deichmann et al. (1944) reported marked systemic toxicity in rabbits and deaths in guinea pigs at exposure concentrations that caused no histopathology in rats. No other inhalation studies in guinea pigs or rabbits have been conducted to confirm these findings. In addition, it is unclear which of these species is most like humans.

The primary data need in order to develop an RfC is a 90-day inhalation study that includes a thorough examination of the respiratory tract. Pharmacokinetic studies of inhaled phenol would also aid in the extrapolation from experimental animals to humans.

6.1.3 Cancer

Several epidemiology studies have evaluated the carcinogenesis of phenol, but have not found a consistent dose-related association. Because all of the subjects were also exposed to other chemicals, and there was no correction for smoking, these studies are not adequate to reach conclusions on the carcinogenic potential of phenol.

Phenol was negative in drinking water bioassays with rats and mice (NCI, 1980), although an increased incidence of leukemias was observed in low-dose male rats. No inhalation studies of sufficient duration to assess carcinogenicity were found. In short-term dermal assays, tumorigenicity (production of papillomas in the absence of treatment by an initiating agent) was observed only at a dose/concentration combination that produced ulceration, and thus was well above the maximal tolerated dose (MTD) (Salaman and Glendenning, 1957). Similarly, although

phenol was a promoter when tested in initiation/promotion studies, the doses tested typically caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959) and death (Boutwell and Bosch, 1959). There were two exceptions. First, the low concentration tested by Salaman and Glendenning, 1957) caused promotion as well as "transient light crusting." Since the site of the weekly treatment was rotated across four sites on the body, it is unclear whether more severe effects would have been observed if the same site had been treated for the entire study. The second exception was that the low concentration tested by Wynder and Hoffmann (1961) was reported as causing no toxicity, although the sensitivity of the evaluation is unclear. Based on the high observed toxicity, it is not clear if the observed promoting activity observed for phenol in several studies was secondary to the repeated injury and healing of the skin. Based on these considerations, the human carcinogenic potential of phenol *cannot be determined*, due to inadequate data.

6.2 Dose-response

No human data that are adequate for the derivation of a phenol RfD were located. Therefore, laboratory animal data were used.

The RfD of 0.6 mg/kg-day was based on decreased fetal body weight and decreased maternal body weight in gavage rat developmental toxicity studies (NTP, 1983a; Argus Research Laboratories, 1997), with a NOAEL of 60 mg/kg-day. There was a corresponding *equivocal* LOAEL for developmental toxicity and a LOAEL for maternal toxicity (not equivocal) of 120 mg/kg-day in these two studies, respectively. A composite UF of 100 was used. This is based on a default factor of 10 for extrapolation from laboratory animals to humans, and a default factor of 10 to account for intrahuman variability. This RfD is several times higher than the endogenous rate of phenol formation in humans of 0.014 - 0.14 mg/kg-day (as summarized by Health Canada, 1999). Note also that the RfD is meant to be for phenol ingested *in addition to* the endogenous formation of phenol.

The principal studies used an adequate number of animals and evaluated an appropriate array of endpoints for a developmental toxicity study. Although both of these studies used gavage dosing, the Argus Research Laboratories (1997) used a divided dosing protocol, a significant enhancement that made the gavage dosing more closely resemble an environmentally relevant route of exposure. Although systemic toxicity of phenol administered by gavage is greater than that of phenol administered in drinking water, it is uncertain whether the developmental toxicity is also greater from the gavage treatment. The principal studies are judged together to have medium confidence. Although the use of gavage dosing lowers the confidence in the studies, the confidence is raised by the consistency of the NOAEL when a divided dosing protocol was used. Confidence in the supporting database is also medium to high. Although the oral toxicity database meets the minimal criteria for a high confidence database (chronic studies in two species, developmental toxicity studies in two species, and a multigeneration reproduction study), the chronic studies did not evaluate a sufficient array of endpoints. In particular, the chronic mouse study did not evaluate hematological effects, making interpretation of the results of the Hsieh et al. (1992) study difficult. Considering the above issues results in medium to high confidence in the RfD.

Although a substantial amount of data on phenol toxicokinetics are available, the data are not sufficient to move away from the default UFs for interspecies extrapolation and intraspecies variability. Data on how blood levels of phenol and its metabolites relate to doses in rats and humans would be useful in addressing the interspecies UF, as would data on the potential for phenol to cross the placenta. Similarly, data on how differences in enzyme activities relate to phenol and metabolite blood levels would be useful in addressing intrahuman variability. Finally, a drinking water study of at least subchronic duration that evaluated hematological effects and immunological effects in mice could address the uncertainties associated with the Hsieh et al. (1992) study, and therefore remove the need for a database UF.

The data available to derive a RfC are inadequate. As noted above, a 90-day inhalation study that evaluated the respiratory tract would be necessary for development of an RfC.

Because the data were considered inadequate to assess the carcinogenicity of phenol, no quantitative assessment was conducted.

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Table 2. Summary of Oral Toxicity Studies

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Systemic Toxicity								
F344 Rat 50/sex/ group	NCI ,1980	0, 2500, 5000 ppm; 0, 260, 585 (M), 0, 280, 630 (F) mg/kg-day	Drinking water	103 weeks	Kidney inflammation and decreased body weight (compared to controls) in both sexes. Decreased water consumption at high dose	260	585	Study authors stated there were no noncancer effects, but independent evaluation for this assessment found significant increase in kidney inflammation
B6C3F1 Mouse 50/sex/ group	NCI, 1980	0, 2500, 5000 ppm; 0, 450 or 660 mg/kg-day	Drinking water	103 weeks	Decreased body weight, decreased water consumption	450	660	Effect apparently secondary to decreased water consumption
F344 Rat 10/sex/ group	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 16, 48, 160, 480, 800 (M) 0, 17, 51, 170, 510, 1140 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight decreased water consumption.	480	800	Range-finding for bioassay; effect apparently secondary to decreased water consumption
B6C3F1 Mouse (10/group)	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 25, 75, 250, 450, 500 (M) 0, 26, 78, 260, 468, 520 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight, decreased water consumption.	450	500	Range-finding for bioassay; effect apparently secondary to decreased water consumption
Sprague-Dawley Rat 15/sex/ group	ClinTrials BioResearch Ltd., 1998	0, 200, 1000, 5000 ppm; 0, 18, 83.1, 308 (M) 0, 24.6, 107, 360 (F) mg/kg-day	Drinking water	13 weeks	Decreased motor activity in females, decreased body weight in males and females, decreased water consumption	107	360	Specialized neurotoxicity study. Decreased body weight apparently secondary to decreased water consumption. Unpublished GLP study
CD-1 Mouse 5 M/group	Hsieh et al., 1992	0, 4.7, 19.5, 95.2 ppm 0, 1.8, 6.2 or 33.6 mg/kg-day	Drinking water	28 days	Decreased hematocrit, decreased immune endpoints.	6.2	33.6	Study uncertainties limit strength of conclusions
Rat 10/group Strain & sex NS	Dow Chemical Co., 1945	0, 50, 100 duration adjusted: 0, 35.7, 71.4 mg/kg-day	Gavage Volume NS	6 months, 5 days/wk	Liver and kidney histopathology; mortality in 4/10 at low and high doses	None	35.7	Unpublished study, small group size, incomplete reporting; authors raised questions about the mortality, although it is unclear what the issue was

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
F344 Rat 8 F/group	Berman et al., 1995; Moser et al., 1995	0, 4, 12, 40, 120 mg/kg-day	Gavage 1 mL/kg	14 days	Tubular degeneration, tremor, increased rearing post-exposure	12	40	Small group size, small dosage volume
Reproductive and Developmental Toxicity								
Sprague-Dawley Rat 30/sex/ group P1; 20/sex/ group F1	IIT Research Institute, 1999	0, 200, 1000, 5000 ppm 0, 14.7, 70.9, 301 (P1 M) 0, 20, 93, 320.5 (P1 F) mg/kg-day	Drinking water	2 genera-tions	Decreased parental and pup body weight, decreased pup survival, decreased water consumption	70.9	301	Study also included evaluation of hematology, serum biochemistry, and developmental landmarks Unpublished GLP study Effects may be secondary to decreased water consumption
Sprague-Dawley Rat 25 F/group	Argus Research Laboratories, 1997	0, 60,120 or 360 mg/kg-day	Gavage 10 mL/kg	GD 6-15	Decreased maternal weight gain; Decreased fetal body weight and delayed ossification	60 (maternal) 120 (dev)	120 (maternal) 360 (dev)	Doses were divided into 3 administrations/day One dam died at 360 mg/kg-day Unpublished GLP study
CD Rats 20-22 F/group	NTP, 1983a	0, 30, 60 or 120 mg/kg-day	Gavage 5 mL/kg	GD 6-15	Decreased fetal body weight	120 (maternal) 60 (dev)	None (maternal) 120 (dev)	None Developmental LOAEL is equivocal
CD Rat 5-10 F/group	NTP, 1983a	0-250 mg/dg-day	Gavage 1-7.5 mL/kg	GD 6-15	Toxicity (tremors, liver and lung pathology, death) markedly higher in smaller dosing volume.	N/A	N/A	Range-finding studies.
CD-1 Mouse, 31-36 F/group	NTP, 1983b	0, 70, 140 or 280 mg/kg-day	Gavage 10 mL/kg	GD 6-15	4/36 dams died, tremors, reduced maternal body weight (10%); reduced fetal body weight	140 (maternal) 140 (dev)	280 (maternal FEL) 280 (dev)	None
F344 Rat 15-20 F/group	Narotsky and Kavlock, 1995	0, 40, 53.3 mg/kg-day	Gavage 1 mL/kg	GD 6-19	Maternal rales and dyspnea, marginal decreases in maternal body weight	N/A	N/A	Screening study

NS = Not Stated; dev = developmental

Table 3. Total Activity Counts in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Dose Group	Prestudy (Mean ±SD)	Week 4 (Mean ±SD)	Week 8 (Mean ±SD)	Week 13 (Mean ±SD)
Females				
Control	384±116	468±118	436±75	309±77
200 ppm	386±89	451±149	440±99	338±66
1000 ppm	384±103	394±78	436±104¶	343±124¶
5000 ppm	372±142	337±127**	363±111¶¶	366±145¶¶
Males				
Control	354±109	339±89	320±90	260±68
200 ppm	340±107	346±132	323±88	256±78
1000 ppm	335±126	356±137	359±105	274±103
5000 ppm	277±59	321±95	352±91	275±116

**Significantly different from control, $p<0.01$ (T-test)
¶Linear constructed variable significantly different from control, $p<0.05$ (T-test)
¶¶Linear constructed variable significantly different from control, $p<0.01$ (T-test)

Table 4. Individual Data on Dehydration and Week 4 Motor Activity in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1501	383	No	2501	397	No	3501	322	No	4601	501	14, 21
1502	321	No	2502	529	No	3502	402	18, 21	4502	No data - sacrificed day 14 due to poor condition	
1502	621	No	2503	427	No	3603	270	No	4503	227	7, 14, 21, 28
1504	437	No	2504	558	No	3504	370	No	4504	258	14, 21, 28, 35, 42, 70
1505	630	No	2505	245	No	3505	572	21, 28, 35, 42, 49, 56, 70	4505	396	No
1506	365	No	2506	537	No	3506	452	No	4506	277	70
1507	591	No	2507	470	No	3507	461	No	4507	399	7-9, 11, 12, 13-15, 17, 20, 21, 70, 77
1508	318	No	2508	284	No	3508	342	No	4508	271	No
1509	479	No	2509	527	No	3509	462	No	4509	387	No
1510	469	No	2510	823	No	3510	452	No	4510	450	No
1511	309	No	2511	561	No	3511	390	No	4511	439	7

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1512	574	No	2512	424	No	3512	383	No	4512	130	No
1513	566	No	2513	302	No	3513	320	No	4513	242	49, 56
1514	381	No	2514	289	No	3514	403	No	4514	180	7, 14, 21, 28
1515	578	No	2515	386	No	3515	311	No	4515	556	No
Overall Average	468	--		451	--		394	--		337	--
Average - Dehydrated animals	N/A	--		N/A	--		487 (n=2)	--		315	--
Average - non-dehydrated animals	468	--		451	--		380	--		365	--

N/A = Not applicable

Table 5. Effects of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters in Mice[a]

Dose (mg/L)	Dose (mg/kg-day)	Spleen cellularity	WBC[b] (x 10^{-3})	RBC[b] (x 10^{-6})	Hematocrit %	Differential counts as % of WBCs		
						Lymphocyte	Neutrophil	Monocyte
Hsieh et al. (1992) - 4-week study in mice								
0.0	0	8.59 ± 0.34	6.06 ± 0.17	7.17 ± 0.56	48.00 ± 0.52	74.20 ± 1.83	17.00 ± 1.00	4.60 ± 0.51
4.7	1.8	7.94 ± 0.20	5.82 ± 0.60	4.90 ± 0.54[c]	49.10 ± 0.68	71.80 ± 2.06	19.40 ± 0.75	4.80 ± 1.02
19.5	6.3	7.31 ± 0.40	5.05 ± 0.53	4.64 ± 0.76[c]	48.20 ± 1.24	69.20 ± 3.25	21.80 ± 2.40	4.60 ± 0.81
95.2	33.6	7.26 ± 0.55	5.68 ± 0.69	3.23 ± 0.68[c]	44.10 ± 0.81[c]	73.60 ± 2.32	17.00 ± 1.55	6.20 ± 1.16
Historical control value[d]		Not available	9.0 (8.9-9.1)	7.6 (7.2-8.0)	42 (36-48)	70 (52-86)	25 (10-42)	4 (0-8)
IIT Research Institute, 1999 - 2-generation study in rats								
Dose (mg/L)	**Dose (mg/kg-day)**	**Spleen cellularity**	**WBC[e] thsn/cmm**	**RBC mill/cmm**	**Hematocrit %**	**Differential counts as % of WBCs**		
						Lymphocyte	**Neutrophil**	**Monocyte**
0	0	N/A	13.1±2.01	9.22±0.37	46.5±1.44	N/A	N/A	N/A
200	15	N/A	13.8±1.98	9.08±0.62	46.2±3.65	N/A	N/A	N/A
1000	71	N/A	14.5±2.42	9.03±0.34	46.4±1.56	N/A	N/A	N/A
5000	301	N/A	14.9±2.93	8.81±0.44	45.1±1.75	N/A	N/A	N/A

[a]Values are given as mean ± S.E. (n=5). Data from Hsieh et al., 1992, unless otherwise indicated. [b]Cells/mm^3. [c]Significant ($P < 0.05$) difference from the control value. [d]Mean (Range ±2S.D) for mice 6-8 weeks of age, based on 20 studies, from Charles River Laboratories, (1986). [e]Mean ±SD

Table 6. Summary of Inhalation Toxicity Studies

Strain Species, Sex	Reference	Exposure mg/m^3	Duration	Duration-Adjusted mg/m^3	Response	NOAEL/ LOAEL mg/m^3	NOAEL/ LOAEL (HEC) mg/m^3	Comments
F344 Rat (20/sex)	Huntingdon 1998	0, 2.0, 18.9, 96.2 Nose-only	6 hr/d 5 d/week 2 weeks	0, 0.36, 3.4, 17	Red nasal discharge, but no histopathology lesions.	0.36/3.4	0.05/0.5	Well-conducted study, but authors did not note the clinical signs Unpublished GLP study
Rat, 7 exposed, 11-12 controls (Strain & sex NS)	Dalin and Kristoffersson 1974	0, 100	15 days continuous	0, 100	Nervous system effects, increased serum liver enzymes	None/100	None/100	Exposure measurement not done according to modern methods, no histopathology exam
Guinea pig, 12 (Strain & sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 6 weeks	31 (based on midpoint of range)	FEL – 5/12 dead	None	None/ 31 is FEL	Minimal documentation, outdated exposure methods, no controls
Rabbit 6 exposed (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 13 weeks	31 (based on midpoint of range)	Pneumonia, heart inflammation, liver necrosis, kidney tubular degeneration	None	None/ 31	Minimal documentation, outdated exposure methods, no controls
Rat, 12 (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 74 days	31 (based on midpoint of range)	No effect, no evidence of histopathology	31/None	31/None	Minimal documentation, sensitivity of assay unclear, outdated exposure methods, no controls
Rhesus monkey 10 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study
Sprague-Dawley rat 50 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study
Albino mouse 100 M/group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Lung pathology (Not further described)	None/18.2	Not determinable*	Pathology reported to be minimal, but limited by minimal description Unpublished study

NS = Not stated

* HEC cannot be determined because the region of the respiratory tract affected is not clear.

Table 7. Key Results from Developmental Toxicity Study in Rats Administered Phenol by Gavage (NTP, 1983a)

	Control	30 mg/kg-day	60 mg/kg-day	120 mg/kg-day
Live fetuses/litter[1]	12.23±0.51	13.32±0.51	12.14±0.56	13.75±0.62
Average fetal body weight per litter (g)	4.14±0.07	4.10±0.05	4.03±0.07	3.84±0.05**
Historical control fetal weight	Mean 3.39 g Range 3.04-3.52 g			
Total ave. fetal weight/dam (g) (calculated)	50.6	54.6	48.9	52.8
Gravid uterine weight (g)	76.9±3.0	82.9±3.4	75.4±3.2	81.7±3.7
Treatment period maternal weight gain (g)	41.0±1.1	47.2±1.7**	40.2±1.6	41.2±2.9
Absolute maternal weight gain (adjusted for gravid uterine weight) (g)	58.0±3.1	58.4±2.2	52.7±2.4	51.8±3.2

[1]Mean ±Standard error of the mean.
**Statistically significant, $p<0.01$

Table 8. Summary of Genotoxicity Studies

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
In Vitro Studies				
Gene mutation-bacteria	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100	-/-	Tested to cytotoxic doses, varying S9 concentrations	Pool and Lin, 1982
	Salmonella typhimurium TA1535, TA1537, TA98, TA100	-/-	Part of NTP testing; tested in 2 laboratories	Haworth et al. 1983
	Salmonella typhimurium TA100	- [1]	Unclear if sufficiently high doses tested	Rapson et al. 1980
	Salmonella typhimurium TA98	-/w [2]	Other strains also tested, but results with them unclear	Gocke et al. 1981
Gene mutation - mammalian cell	Mouse lymphoma L5178Y cells	?/?[2]	Two independent assays conducted +/- S9	McGregor et al. 1988a, 1988b
	Chinese hamster V79 cells	NT[2]/+	S9 from phenobarbital-induced mice	Paschin and Bahitova 1982
	Syrian hamster embryo (SHE) cells	NT/+	None	Tsutsui et al. 1997
Clastogenicity	Micronuclei in human lymphocytes	+/NT	None	Yager et al. 1990
	Micronuclei in CHO cells	+/+	S9 from phenobarbital/beta-naphthoflavone induced rats	Miller et al. 1995
Chromosome aberration	CHO cells	-/+	Part of NTP testing	Ivett et al. 1989
DNA damage	Sister chromatid exchange, human lymphocytes	-/NT	Unclear if sufficiently high doses tested	Jansson et al. 1986

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
	Sister chromatid exchange, human lymphocytes	+/NT	Small increases	Morimoto et al. 1983; Morimoto and Wolff 1980
	Sister chromatid exchange, human lymphocytes	+/NT	None	Erexson et al. 1985
	Sister chromatid exchange, CHO cells	+/w	Part of NTP testing	Ivett et al. 1989
	Unscheduled DNA synthesis, SHE cells	+/NT	None	Tsutsui et al. 1997
	Single strand breaks mouse lymphoma cells	-	None	Pellack-Walker and Blumer, 1986
	Single strand breaks CHO cells	-	None	Sze et al. 1996
Cell transformation	Syrian hamster embryo (SHE) cells	+/NT	None	Tsutsui et al. 1997
In Vivo Studies				
Gene mutation	*Drosophila* sex-linked recessive lethal	-	None	Gocke et al. 1981
	Drosophila sex-linked recessive lethal	-	None	Sturtevant 1952
	Drosophila sex-linked recessive lethal	-	None	Woodruff et al. 1985
Clastogenicity	Mouse micronucleus i.p.	+	None	Marazzini et al. 1994
	Mouse micronucleus i.p.	+	Weak response	Chen and Eastmond 1995a
	Mouse micronucleus i.p.	-	No positive control, unclear if sufficiently high doses tested	Barale et al. 1990

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
	Mouse micronucleus i.p.	-	Small sample size	Gocke et al. 1981
	Mouse micronucleus oral	w	None	Ciranni et al. 1988b
	Mouse micronucleus i.p.	+	Clear effect at same dose as oral study	Ciranni et al. 1988b
	Mouse micronucleus oral	-	Number tested not reported	Gad el-Karim et al. 1985
	Mouse micronucleus oral	+	Pregnant females on GD13	Ciranni et al. 1988a
Chromosome aberration	Mouse, spermatogonia and spermatocytes	+	Inconsistencies in reporting	Bulsiewicz 1977
DNA damage	Single strand breaks, testicular cells, i.p.	-	None	Skare and Schrotel 1984

[1]Apparently in the absence of S9 - the presence of absence of S9 was not addressed.
[2]w = weak positive response; ? = questionable or inconclusive; NT = not tested

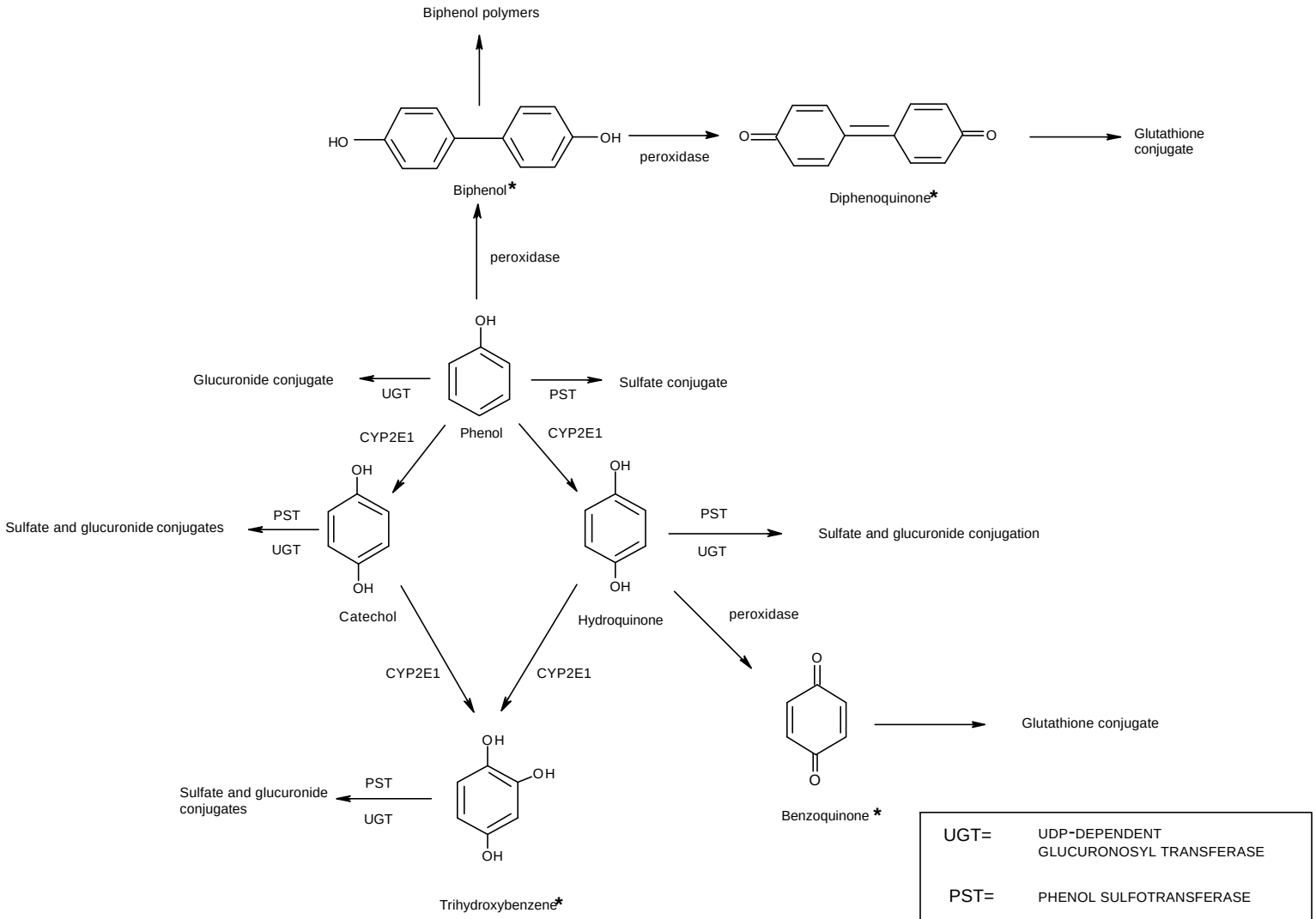
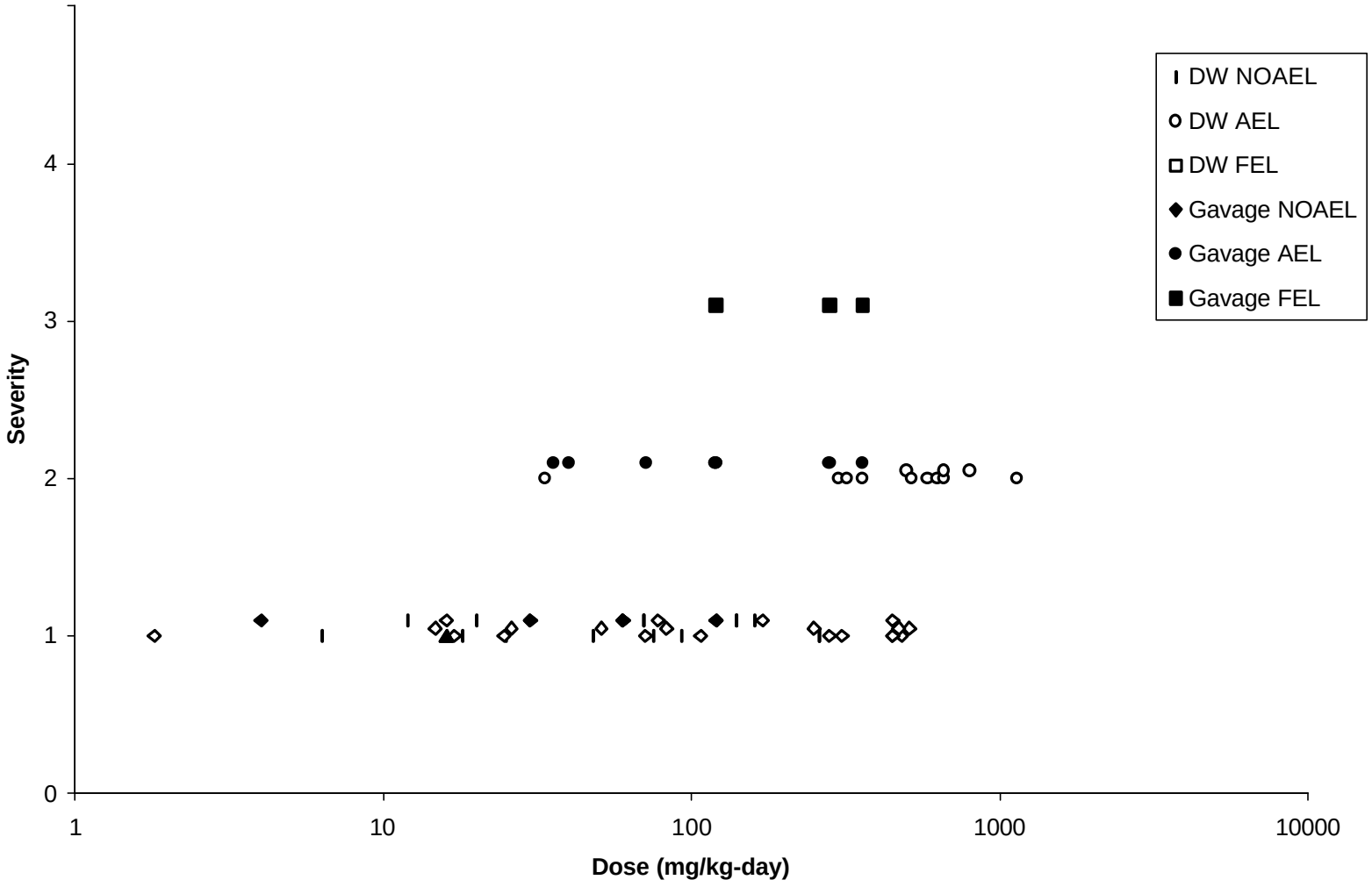


Figure 1. Metabolism of Phenol.

Figure 2. Plot of severity with dose for drinking water (DW) (open symbols) or gavage (filled-in symbols). Values of 1, 2, 3 correspond with NOAEL, AEL, or FEL, respectively.



8.0 APPENDICES